Original Article

Incidence of Recurrent High Grade Cervical Intraepithelial Neoplasia after Treatment of Preinvasive Cervical Lesion: A-3 Year Follow-up at Thammasat University Hospital

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Background: Cervical cancer is the third most malignancy among Thai women. Cervical intraepithelial neoplasia(CIN) 2/3 is precancerous of cervical cancer that could be conservatively treated. A precise recurrent investigation tool is an important method.

Objective: The aim of this retrospective study is to investigate the regression and recurrence rate of CIN2/3 after three years of treatment.

Materials and Methods: Ninety cases of CIN2/3 from participants who underwent cervical cancer screening and treated between July 2013 and June 2016 at Gynecologic Clinic, Thammasat University Hospital, Pathumthani, Thailand and affiliated clinics were recruited. Demographic data, method of treatment and co-testing report of participants were recorded for three consecutive years.

Results: A total of 2,144 women during the period were recruited. After those excluded, 1,505 cases were enrolled in this study. Cases who had abnormal cervical cytology had less mean age, more sexual partners and sexual transmitted diseases than those who had normal cervical cytology. Ninety-nine percent of the negative testing group had negative co-testing at the end of the three-year period. Ninety cases were diagnosed with CIN2/3. The regression rates of CIN2/3 at two and three years follow-up after treatment were 97.8% (88/90) and 95.6% (86/90), respectively. An additional four cases that had negative co-testing at first and second year were diagnosed of CIN2/3 at third year of follow-up period.

Conclusion: The regression and recurrence rates of CIN2/3 at three-year follow-ups are 95.6 and 4.4 percent, respectively. Over-extending by three consecutives co-testing follow-ups of CIN2/3 after treatment should be of concern.

Keywords: CIN, Pap smear, High grade, Follow-up, Excision procedure, Ablation

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According to the National cancer institute of Thailand, cervical cancer is the third most occurring malignancy among Thai women. The age-standardized incidence rate (ASR) is as high as 16.2 per 100,000 Thai female population (Globalcan 2018). The responsible pathogen for cervical cancer is proven to be the human papillomavirus (HPV). Nowadays, the incidence rate of cervical cancer is declining by potent and sophisticated screening and referring system. There are various HPV testings combined with cervical cytology are currently popular cervical cancer screening methods. HPV testing is divided into DNA and mRNA method that of the HPV DNA testing offered higher sensitivity but lower specificity than HPV mRNA testing[(1)]. The HPV DNA testing yielded high stability of DNA fragment that could be detected in both recent or latent HPV infection from cervical scraping specimen.

On the other hand, the HPV mRNA testing offers appropriate sensitivity in identifying higher CIN stages at appropriate disease timelines compared to the HPV DNA method. During the process of HPV infection, the presence of mRNA is ephemeral. The detection of the HPV mRNA indicated that there is a recent HPV infection. The current mRNA HPV testing checks E6/E7 location of the HPV genome which certainly represents a tight correlation with cervical cancer incidence. Thammasat University Hospital has been offering the HPV mRNA testing since 2013.

Recent of abnormal cervical cytology, could be classified into low and high risk groups. Low risk group is comprised of an atypical squamous cell of undetermined

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(ASCUS) and low grade squamous intraepithelial lesion (LSIL). The high risk group accounts for atypical squamous cannot exclude high grade squamous intraepithelial lesion (ASCH), high grade squamous intraepithelial lesion (HSIL) and atypical glandular cell (AGC).

According to ASCCP guideline 2014, the management of abnormal cervical cytology would be different according to the aggression, as identified by the prior severity of the abnormal cervical cytology. Cervical intraepithelial neoplasia 2/3 (CIN2/3) was normally treated by either the ablation or excision procedure. CIN1 patients mostly recieved close followed-up or underwent minimal invasive procedure[2]. After the completion of CIN2/3 treatment, annual co-testing (cervical cytology plus HPV testing) is recommended for two consecutive years. Cases with equal or less than CIN1 should be followed-up with cotesting in one year. Once negative cotesting was achieved at the end of follow-up period, patients would undergo normal routine cervical cancer screening.

The aim of this retrospective study is to investigate the prevalence of CIN2/3 after three years post treatment. Factors influencing the recurrent and persistent high grade CIN2+ in women with abnormal cotesting would be investigated.

Materials and Methods

This retrospective review study was performed at the Department of Obstetrics and Gynecology, Thammasat University Hospital, Pathum Thani, Thailand. It was approved by the Ethic committee of Faculty of Medicine Thammasat University prior to the study (MTU-EC-OB-1-205/61).

Patient records of those who underwent cervical cancer screening by co-testing method between July 2013 and June 2016 were retrieved and compiled. Participants who had normal cervical cytology and HPV testing were recommended to undergo triennial co-testing (liquid based cervical cytology plus HPV testing). Those who had abnormal cervical cytology or HPV testing were managed according to ASCCP guidelines. During the period of study, HPV mRNA testing was performed by using commercial kit (Aptima®, California, USA). Patients who were lost to follow-up during the period, had incomplete medical records or became cervical cancer patients, were otherwise excluded. Cases with abnormal cervical cytology from affiliated clinics, who were referred to Thammasat University Hospital for standard treatment and follow-up, were also included.

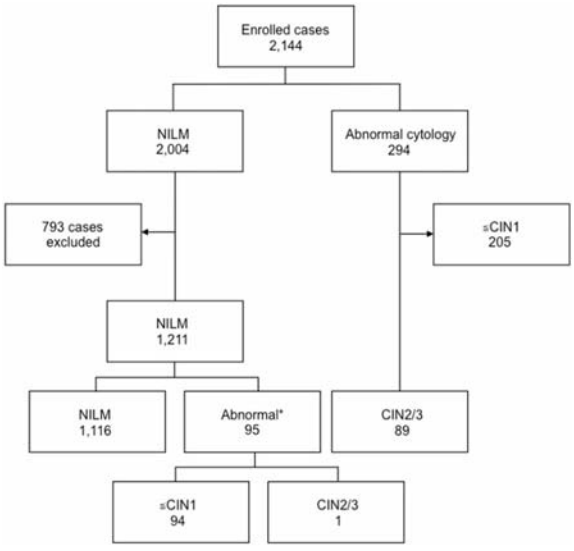
Information database reviewed from medical records, both written documents and computerized database, included result of cytology, HPV testing (16, 18/45 and other types) at the first and three years follow-up. Result of the colposcopic examination and histopathological reports were reviewed. Furthermore, additional data were also collected namely age, parity, abortion, number of sexual partners, contraception, menopausal status and HPV vaccination.

Patient demographic data were treated with descriptive statistic. Median, mean and standard deviation (SD) were used for continuous data while Chi-square or Fisher's exact test were used for categorial data. All statistics were analysed by SPSS Statistics version 23 (SPSS Inc., Chicago, USA) and a *p*-value of less than 0.5 were consider significant.

Results

During the period of study, a total of 2,144 women came to gynecological clinic at Thammasat University Hospital for cervical cancer screening by co-testing. Ninety cases were diagnosed with CIN2/3. Seven hundred and ninety three cases fall into the exclusion criteria as presented in Figure 1. Cases with negative co-testing at the period of recruitment all had co-testing repeated three years interval. Ninety-nine percent (1,202/1,211) of the negative testing group had negative co-testing at the end of three years period. Table 1 presented demographic characters of participant at the beginning of the study. Participants were divided into two groups, namely abnormal (A) and normal (B) cervical cancer screening. Participant in group A had lesser mean age, parity and contraceptive usage than group B with statistical difference. Number of sex partners, Human Immunodeficiency Virus (HIV) and Sexual Transmitted Diseases (STDs) infection were statistically higher than that of group B. Participants in both groups received post exposure HPV vaccination.

Data showed 1,211 cases with negative result for intraepithelial lesion or malignancy (NILM) cytology. There were 95 cases with positive result in high risk HPV testing.



* NILM with HPV high risk types positive.
NILM = negative for intraepithelial lesion or malignancy, CIN = cervical intraepithelial neoplasia, HPV = human papillomavirus

Figure 1. Flow chart of this study.

Twenty-three cases of those with positive high risk HPV testing had positive HPV type 16/18. All 23 cases of NILM cervical cytology with positive HPV type 16/18 underwent colposcopic directed biopsy. Only 4.3% (1/23) cases were diagnosed with CIN2/3. They later underwent cervical ablation treatment.

There are 389 cases with abnormal cervical screening, including negative for intraepithelial lesion of malignancy (NILM) with positive high risk HPV 95 cases and abnormal cervical cytology 294 cases. All of 389 cases underwent colposcopic directed biopsy. CIN2/3 was diagnosed in 90 cases. There are 22 and 67 cases from participants who had low grade and high grade cervical cytology, respectively. And only one case from NILM with positive HPV type 16/18 as presented in Table 2.

Among 90 cases with CIN2/3 in this study, the regression of high grade lesion at follow up after one and two years were 93.3% (84/90) and 97.8% (88/90), respectively as showed in Figure 2. The persistent high grade lesion found at one and two years follow-up were at 6.7% (6/90) and 2.2% (2/90), respectively. Four cases were diagnosed of CIN2+ at the third year follow-up while they were all of free disease during the first and second year follow-up. Furthermore, there is only one case with positive result of high risk HPV for two consecutive years while 3 others presented with negative results in the second year.

All 4 cases with recurrent CIN2/3 at 3 years follow-up had positive high risk HPV. All cases immediately underwent cervical ablation, because of fertility requirement and small lesion (0.3*0.2*0.2 cm), and excision as presented in Table 3. Two consecutive years of co-testing in this group showed result in all patients. All persistent and recurrent CIN2/3 cases had no history of HPV vaccination.

Table 1. Demographic data of enroll cases during study period

	Cervical cytology, n (%)		*p*-value
	Positive (n = 294)	Negative (n = 1,211)	
Age (year)*	47±11.3	51.7±9.6	<0.001
Multiparity	185 (62.9)	971 (80.2)	<0.001
Abortion	30 (10.2)	143 (11.8)	0.43
Polygamy	143 (48.6)	251 (20.7)	<0.001
STDs	199 (67.7)	133 (60.5)	<0.023
HIV infection	18 (6.1)	17 (1.4)	<0.001
Menopause	122 (41.5)	664 (54.8)	<0.001
Vaccination	52 (17.7)	280 (23.1)	0.22
Contraception			
Hormonal	55 (18.7)	365 (30.1)	<0.001
Barrier method	60 (20.4)	189 (15.6)	<0.001

* mean ± SD (standard deviation)
STDs = sexual transmitted diseases, HIV = human immunodeficiency virus, Vaccination = bivalent or quadrivalent of HPV vaccine, HPV = human papillomavirus, Positive = abnormal cytology, Negative = normal cytology

Discussion

Data in the present study was collected from women who underwent cervical cancer screening at Thammasat University Hospital between July 2013 and June 2016. Cases with CIN2/3 diagnosis either from conventional or liquid based cervical cytology were treated according ASCCP guideline. A three years follow-up was checked and result was evaluated. After the case selection and the exclusion process (Figure 1), 1,505 cases were chosen. Positive cervical cytology was found at 19.5% (294/1505). Women who had abnormal cervical cytology were significantly younger with higher number of sexual partners than those with normal cervical cytology ($p \leq 0.05$). Thai traditional belief had taught women to value themselves and withhold sex until their marriage. Nowadays, sexual behavior of young women has changed from the past. Age of first sexual intercourse among young women in the education system went down from 17.6 to 16.7 years from 2003 to 2011 and the average amount of partners increased from 2.6 to 3.5[3,4]. Young people infrequently use contraceptive and sex was usually unprepared thus they were unprotected and lead to more STDs infection[3]. Multiparity, polygamy and STDs, were associated with HPV infection[5].

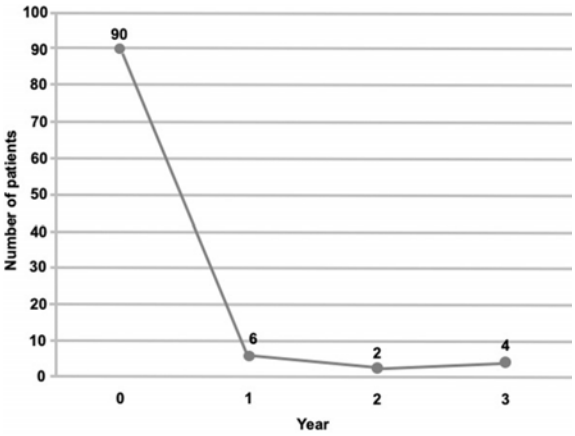
Cases with CIN2/3 mostly underwent excisional treatment procedure (80/90) with 67.5% (54/80) free margin. The remaining cases underwent cryotherapy (10/90). Two years remission rate in the present study was 97.8% (88/90). The comparison of persistent, recurrent and regression rate of CIN 2/3 in various studies were summarized and represented in Table 4. Wu and coworker reported a Chinese study in 2015 that two year regression rate of CIN2/3 was 97 percent[2]. Our two years regression was 97.8. All cases in Wu's study underwent excisional treatment procedure

Table 2. Cervical cytology, pathology and treatment procedure of cases during study period

Cytology	CIN2/3*		
	No intervention	Ablation	Excision
NILM (n = 1,211)	0	1 (1.1)	0
Low grade (n = 188)	0	2 (2.2)	20 (22.2)
High grade (n = 106)	0	7 (7.8)	60 (66.7)

* n (%)
NILM = negative for intraepithelial lesion or malignancy, CIN = cervical intraepithelial neoplasia, Low grade = ASC-US and LSIL, High grade = ASC-H, HSIL and AGC, ASC-US = atypical squamous cell of undetermined, LSIL = low grade squamous intraepithelial lesion, ASC-H = atypical squamous cannot exclude high grade squamous intraepithelial lesion, HSIL = high grade squamous intraepithelial lesion, AGC = atypical glandular cell, No intervention = no treatment procedure, Ablation = cryotherapy or laser, Excision = loop electrical excisional procedure or conization

compared to 90% in the present study. The percentage of free margin in Wu's study was 87.4 while our current investigation was 67.5. Since this investigation had 10% ablation this group would not yield free margin result. Wu's study used the same liquid based cytology (Thinprep®) tool as our study. The HPV testing in Wu's study was PCR based while E6/E7 detection by mRNA was utilized by our lab.



CIN = cervical intraepithelial neoplasia

Figure 2. Cumulative number of CIN2/3 cases after 3 years follow-up.

Mistro et al 2015 work in Italy reported a 91.2 percent CIN2/3 one year remission rate[6]. All CIN2/3 cases in Mistro series underwent excisional procedure with negative margin rate of 66.6 percent. The rate was comparable to the result of the present study. Mistro's follow-up in Mistro's study includes either liquid based cytology or HPV co-testing with household PCR.

Moore et al from USA and Lili et al from Greece in 2007 and 2018 both used conventional Pap smear for post treatment after excisional procedure[7,8]. Moore's and Lily's study reported 84 and 99 percent of remission at 3 years, respectively. The sensitivity in disease detection in Moore and Lili's works was lower than that of the current finding. This is in line with the knowledge that conventional Pap smear sensitivity is expected to be lower than that of the co-testing[9]. The high number of undetectable diseases might contain the silent disease (false negative rate).

Moscicki et al work in 2010 reported three years regression rate of CIN2/3 after all excision treatment at 68 percent[10]. This current study utilized the same Pap smear commercial kit with Moscicki's study but we used E6/E7 mRNA HPV detection while Moscicki used DNA PCR. This finding yielded a higher regression rate compared to Moscicki's work because mRNA HPV testing yields a better result

Table 3. Characteristic of CIN2/3 patients with recurrent disease after follow-up 3 years

Number	Age (years)	Initial diagnosis	HPV testing	Treatment	Recurrent diagnosis
1	35	CIN3	+HR, +type 16/18	Cryotherapy	CIN3
2	48	CIN3	+HR, -type 16/18	LEEP	CIN2
3	70	CIN3	+HR, +type 16/18	LEEP	CIN3
4	78	CIN3	+HR, -type 16/18	LEEP	CIN3

CIN = cervical intraepithelial neoplasia, HPV = human papillomavirus, +HR = positive for HPV high risk group, +type 16/18 = positive for HPV type 16/18, -type 16/18 = negative for HPV type 16/18, LEEP = loop electrosurgical excisional procedure, all resection margins are negative

Table 4. Comparison of persistent, recurrent and regression rate of CIN2/3 in various studies

Study	Present study	Lili	Mistro	Jin Wu	Katki	Moscicki	Moore
Year	2019	2018	2015	2015	2013	2010	2007
Country	Thailand	Greece	Italy	China	USA	USA	USA
Number	90	804	760	836	190	95	177
Type of testing	Co	CPP	Co	Co	CPP, Co	Co	CPP
HPV testing	mRNA	-	DNA	DNA	DNA	DNA	-
F/U (Year)	3	3	1	1	5	3	2
CIN2/3 (%)							
Persistent	2.2-6.7*	-	5.1	-	-	-	11
Recurrent	4.5	1.1	3.7	2	16	15	5
Regression	93.3-97.8	-	91.2	97	-	68	84

* Follow-up 1 to 2 years

CIN = cervical intraepithelial neoplasia, F/U = period of follow-up, Co = co-testing, CPP = conventional pap smear, HPV = human papillomavirus, mRNA = messenger ribonucleic acid, DNA = deoxyribonucleic acid

compared to DNA method[9].

ASR incidence of cervical cancer in USA, Italy, Greece and China were 6.4 to 10.7 per 100,000 people[2,6-8,10]. The ASR in a recent Thailand report was 16.2 per 100,000 people (Globalcan 2018). Theoretically, higher ASR incidence rate leads to higher number of new cases and recurrent. But our work showed comparable regression to the results from nations with much lower ASR. Our tight yearly follow-up during the first three years after the treatment might be one of the game changer and thus, increasing the regression rate.

HPV vaccine had been introduced to the market since year 2006. All participants in the current study reported no knowledge of HPV vaccination prior to their illnesses. The benefits of HPV vaccination were given to all CIN2/3 cases in their routine outpatient visit. As a result, 1/3 of participants chose to receive post-exposure HPV vaccination. Tendency of the regression, persistent, and recurrent rate were improved considering studies in past decades when compared with the recent studies. Widespread HPV vaccination and more reliability of cervical cancer screening program might also be contributing factors.

All persistent and recurrent cases had no history of post-exposure HPV vaccination. HPV vaccination probably effected the regression of the disease. This was supported by Ghelardi's report of the risk reduction of CIN relapse rate by HPV vaccination of 81.2 percent[11].

ASCCP guideline for post CIN2/3 treatment recommended the follow-up by co-testing for the first, second, and the next 3 years with routine screening if the first and second year visit gave negative testing. In our clinic, most participants with CIN2/3 diagnosis were treated and asked to undergo co-testing for 3 consecutive years. Four point four percent (4/90) were found with recurrent CIN2/3 with negative co-testing result in the first two years of follow-up. Percent of recurrent rate of CIN2/3 at third year follow-up was 1.1 in Lili's study. It looked like surrounding circumstances effected a higher recurrent rate in Thailand than in Europe. From our finding, annual follow-up by co-testing for three consecutive years are highly recommended instead of three year testing interval after negative testing in the second year after the treatment as recommended by ASCCP.

The limitation of the present study was the small sample size of participants. However, this study reported the recurrent rate of CIN2/3 after treatment by liquid based and HPV mRNA testing that had never been reported in Thailand.

Conclusion

The regression and recurrence rates at three years follow-up were 95.6 and 4.4 percent, respectively. Overextending by three consecutives co-testing follow-up of CIN2/3 after treatment should be of concern.

What is already known on this topic?

After the completion of CIN2/3 treatment, annual co-testing (cervical cytology plus HPV testing) was recommended for two consecutive years. When negative cotesting of two consecutives were achieved then routine screening was performed. HPV testing was performed by either DNA or mRNA detection. Detection of mRNA in Thailand for recurrent detection was not previously reported

What this study adds?

In the area of higher incidence HPV infection like Thailand, Overextending by three consecutives co-testing follow-up of CIN2/3 after treatment should be concerned. Life long follow-up of cervical cancer screening should be considered in women who had history of CIN2/3.

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Potential conflicts of interest

The authors declare no conflicts of interest.

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อัตราการกลับเป็นซ้ำของภาวะก่อนมะเร็งปากมดลูกระดับ 2 ถึง 3 ภายหลังการรักษา: ประสบการณ์การตรวจติดตาม 3 ปี ณ โรงพยาบาลธรรมศาสตร์เฉลิมพระเกียรติ

กนกวรรณ พรมชิตร์, อวัสดา บุณยัษเฐียร, ณัฐภัทร กาญจนวิไล, จรรยา ภัทรอาชาชัย, กรณ์กาญจน์ ภมรประวัติธนะ, คมสันติ์ สุวรรณฤกษ์

ภูมิหลัง: มะเร็งปากมดลูกพบเป็นอันดับที่สามของอุบัติการณ์โรคมะเร็งในสตรีไทย ภาวะก่อนมะเร็งปากมดลูกระดับ 2 ถึง 3 (CIN2/3) เป็นภาวะที่มีความเสี่ยงสูงต่อการเกิดมะเร็งปากมดลูก สามารถรักษาแบบอนุรักษ์ได้ ทั้งนี้ต้องมีเครื่องมือที่ถูกต้องและแม่นยำในการตรวจติดตาม

วัตถุประสงค์: เพื่อสืบค้นอัตราการหายและเกิดซ้ำของภาวะก่อนมะเร็งปากมดลูกระดับ 2 ถึง 3 เมื่อตรวจติดตามหลังการรักษา 3 ปี

วัสดุและวิธีการ: ศึกษาสตรีที่มีภาวะก่อนมะเร็งปากมดลูกระดับ 2 ถึง 3 รวมทั้งสิ้น 90 คน ที่ได้รับการตรวจคัดกรองมะเร็งปากมดลูก และรักษาที่โรงพยาบาลธรรมศาสตร์เฉลิมพระเกียรติ ร่วมกับสตรีที่ตรวจพบความผิดปกติ ของเซลล์ปากมดลูกที่ถูกส่งตัวมารักษาจากสถานพยาบาลอื่น ตั้งแต่เดือนกรกฎาคม พ.ศ. 2556 ถึง มิถุนายน พ.ศ. 2559 โดยเก็บข้อมูลทางประชากร, วิธีการรักษา และผลตรวจติดตามด้วยเซลล์วิทยาร่วมกับสารพันธุกรรมของเชื้อไวรัสเอชพีวี เป็นระยะเวลา 3 ปี

ผลการศึกษา: สตรีจำนวน 1,505 คนจากจำนวนสตรีที่เข้ารับการตรวจทั้งหมด 2,144 คน ได้เข้ามามีส่วนร่วมในงานวิจัย ผลการเก็บข้อมูลพบว่าในกลุ่มที่มีผลตรวจเซลล์ปากมดลูกผิดปกติ จะอยู่ในช่วงอายุที่น้อยกว่า มีจำนวนคู่นอนและโรคติดต่อทางเพศสัมพันธ์มากกว่า เมื่อเทียบกับกลุ่มที่มีผลตรวจเซลล์ปากมดลูกปกติ โดยร้อยละ 99 ของสตรีที่มีผลตรวจคัดกรองมะเร็งปากมดลูก ปกติในปีแรกยังคงปกติที่ระยะการตรวจติดตาม 3 ปี สตรีที่มีภาวะก่อนมะเร็งปากมดลูกระดับ 2 ถึง 3 จำนวน 90 คน ซึ่งพบว่ามีอัตราการหายของภาวะก่อนมะเร็งปากมดลูกระดับ 2 ถึง 3 ที่ 2 และ 3 ปี คิดเป็นร้อยละ 97.8 และ 95.6 ตามลำดับ และพบอัตราการเกิดซ้ำของภาวะก่อนมะเร็งปากมดลูกระดับ 2 ถึง 3 ที่ 3 ปีเมื่อตรวจติดตามหลังการรักษา 4 ราย

สรุป: อัตราการหายและการเกิดซ้ำของภาวะก่อนมะเร็งปากมดลูกระดับ 2 ถึง 3 ในการตรวจติดตามที่ 3 ปี หลังการรักษาเป็น ร้อยละ 95.6 และ 4.4 ตามลำดับ ดังนั้นการตรวจติดตามภาวะก่อนมะเร็งปากมดลูกระดับ 2 ถึง 3 หลังการรักษาอาจต้องใช้ระยะเวลานานมากขึ้นโดยเฉพาะในพื้นที่ที่มีอุบัติการณ์การเกิดมะเร็งปากมดลูกสูง