### Prevalence of High-Risk Human Papillomavirus Infection (HPV) and Correlation with Postmenopausal Hormonal Therapy in Thai Women Aged More Than 45 Years Old

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**Objective:** To determine the prevalence of high-risk HPV infection and to evaluate the association between high-risk HPV infection and hormone replacement therapy in Thai women 45 years old and older.

Material and Method: The present cross-sectional study was performed in 600 women aged 45 years old and older who attended the menopausal clinic of Phramongkutklao Hospital. There were 191 hormone users and 392 non-hormone users. The HPV DNA was studied in cellular residual from liquid-based collection tube and analyzed with a hybrid capture two techniques using a mix of high risk viral RNA probe.

**Results:** The prevalence of high-risk HPV infection was 34 (5.67%) of 600 participants. When divided by status of hormone users, the prevalence of this infection in non-users, past user group, and current user group was 5.36%, 4.96%, and 10.00% respectively. There was non-significantly elevated risk among past (adjusted odd ratio (OR) = 0.92, 95% confidence interval (CI) = 0.36-2.34) and current (adjusted OR = 1.96, 95% CI = 0.80-4.81) hormonal users compared to never users. However, women who currently used unopposed estrogen orally had a statistically significant higher risk of high-risk HPV detection (adjusted OR = 9.82, 95% CI = 3.02-31.89).

**Conclusion:** The prevalence of high-risk HPV infection in Thai women 45 years old and older was 5.67%. The oral, unopposed estrogen was associated with a higher incidence of high-risk HPV infection, but a small number of hormonal users. Further investigations are needed.

Keywords: High-risk HPV, High-risk human papillomavirus, Prevalence, Hormone therapy, Postmenopausal

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One of the common causes of death in the global population, beside accidents, is malignancy. Cervical cancer is known to be fatal particularly among the women who are aged between 35-60 years<sup>(1,2)</sup>. Although this is serious, early detection by the annual check-up and identification of high-risk factors that contribute to the cancer development have proven to be an effective means of prevention. Human Papillomavirus (HPV) infection is known to cause cervical cancer, as 99.7% of the pathological specimens is found to be HPV infected<sup>(3)</sup>. There are more than 50 HPV types in the world. However, only 13 of them are

classified as "high-risk" HPVs and are strongly related to the cancer. Previous studies have shown that high-risk HPV infection seems to affect two groups of women: those that are younger than 25 years old and those who are in between the ages of 55-64 years<sup>(4)</sup>. The later group includes the postmenopausal women that have had persistent HPV infection. The prevalence of HPV infection in older women varies but has the same trend that has the second peak of high-risk HPV infection in postmenopausal women<sup>(5-7)</sup>.

Hormonal therapy may also be a risk factor. The commonly used oral contraceptive pills have been found to be a risk factor of HPV infection and cervical cancer in women who are in their fertility age<sup>(8,9)</sup>. However, this remains controversial as this hypothesis is only supported by some studies<sup>(5,10,11)</sup>. Determining

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the impact of the hormonal therapy on HPV infection and cervical cancer is also a great challenge, since there are a limited number of publications in this area.

The aim of the present study was, therefore, to examine the prevalence of the high-risk HPV infection among the Thai women who were older than 45 years and to evaluate the relationship between the high-risk HPV infection and postmenopausal hormonal use.

### **Material and Method**

#### Population sample selection

This cross-sectional study was conducted at Phramongkutklao Hospital from July 2006 to January 2007. The perimenopausal and postmenopausal women aged 45 years old and older, who attended the gynecological out-patient department and the menopausal clinic of the hospital, were enrolled. Women with a history of having a surgical removed cervix were also recruited. The women who had been diagnosed with cancers at other organs were excluded. All participants completed a consent form, which had been approved by the Ethics committee of the hospital. The participants were also requested to answer survey questions and undergo Pap smear examination.

### Data collection

The participants were asked to answer questions regarding their socio-demographics, gynecologic histories, history of hormonal use, and other risk factors of cervical dysplasia and cancer. Sociodemographic questions include their marital status, education, and religion. Gynecologic histories refer to their menopausal status, age of menopause, history of hysterectomy, the age at which they had the first sexual intercourse, the last time they had sexual intercourse, previous contraception used (especially oral combined pills used), the total number of pregnancies, and the total number of sexual partners. History of HPV-related genital lesion (cervical, vaginal and vulvar dysplasia and genital wart), of their smoking habits and of postmenopausal hormonal used (status of use, regimen and route of administration) were also asked. The hormone usages were compared against the patient's medical record. The women whose data was discrepant from the medical record were excluded when determining the relationship between high-risk HPV infection and hormonal usage.

### Pap smear and HPV testing

Pap smear samples were collected as a part of routine gynecological care. The women who had cervix, Pap smear were collected from the posterior fornix of the vagina, ectocervix, and endocervix. For the women who had had a hysterectomy, the cells were collected from the vaginal stump. The Pap smear results were classified based on the Bethesda system as normal or abnormal. The abnormal results were further classified as atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelium lesions (LSIL), high-grade squamous intraepithelium lesions (HSIL) and invasive cancer. The HPV testing was, then, collected using a cervical brush, and immediately suspended in 1.0 mL Cervical Sampler Specimen Transport Medium (Digene Corporation, Gaithersburg, MD, USA). The suspended sample was then frozen until tested.

HPV DNA was tested using a technique called Hybrid Capture 2 (HC2). In this work, HC2 was used to detect the DNA of 13 high-risk HPV types. There are HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. The DNA was released from the cervical cells with an alkaline solution, and hybridized to RNA probes, which are designed for high-risk HPV types. Then, the DNA: RNA hybrids were captured onto the surface of a microtiter well using antibodies specifically designed for hybrid molecules. Alkaline phosphatase was, then, added, followed by chemiluminescent substrate (dioxetane). The fluorescent activity was measured using a luminometer and fluorescent intensity was measured in RLUs (relative light units). A positive result from the HC-2 test is defined as a net fluorescent activity that is greater than or equal to the fluorescent activity average of three manufacture positive controls. The minimum amount of the HPV DNA that gives a positive result is 1.0 pg/mL 1.0 pg is equivalent to 5,000 copies of HPV DNA.

### Statistic analysis

Unpaired t- test was used to compare the data in the HPV-positive with those in the HPV-negative group and Chi-square test was used to test the HPV infection hypothesis. Age-adjusted odds ratios (ORs) and corresponding 95% confidence intervals (CI) were conducted to determine correlation between the HPV infection and the variables. The participants were categorized into two groups: hormonal users and non-hormonal users. The association between HPV detection and potential risk factors was determined by logistic regression, and the association between HPV detection and postmenopausal hormonal usage was determined by multiple logistic regressions. The relationship between HPV infection and hormonal therapy was analyzed based on: the hormonal usage status (never-users, past-users, or current-users), the types of the hormones used (estrogen-only or estrogen/progestin combination), and administration route (oral or transdermal). The relationship was determined in all user groups (never-, past-, and current-users). Then, the results from the past- and the current-users were compared to those from the never-users. The variables with p < 0.05 were chosen for the modeling, which was performed using a forward-stepwise method. All statistical analyses were performed using SPSS version15.

#### Results

# Characteristics of population and high-risk HPV infection

Six hundred women participants were chosen for the present study: 151 perimenopausal women (25.17%) and 449 postmenopausal women (74.83%). The average age of the women (aged from 45-83) was 54.39 with the standard deviation of +7.39 years. The survey results showed that 80.3% of the women were married, and 46.3% had had some post-graduate level education. Other demographic data are shown in Table 1.

The HC2 test detected the high-risk HPV DNA in 34 out of the 600 women (5.67%). 86.67% of the participants had a normal Pap smear (4.2% in the HPV infected group and 95.8% in the non-HPV infected group), and 13.33% of the participants had cervical infection appeared in their Pap smear results (8.1% in the HPV infected group and 91.9% in the non-HPV infected group). The proportions of HPV infected cases in women with and without cervix were 5.16% and 7.76%, and there was no statistical significance. Abnormal Pap smear was found in six of the participants (1.0%). Among these six women, there were one ASCUS, two LSIL, one HSIL, one Adenocarcinoma in situ (AIS), and one squamous cell carcinoma. All of them had identified high-risk HPV infection (100.0%).

No significant correlation of high-risk HPV infection was found with the history of oral contraceptive pills and condom usage. No significant correlation of the high-risk infection associated with the age of the first-time sexual intercourse and the history of having a sexually transmitted disease was found. However, the women who had more than two sexual partners and who were current smokers displayed a higher risk of high-risk HPV infection (adjusted OR = 2.65; 95% confident interval (CI) = 1.18-5.89 and

adjusted OR = 7.66; 95% CI = 1.28-45.65, respectively) as presented in Table 2.

## *Hormone replacement therapy (HRT) route and HPV infection*

583 out of 600 women (97.17%) were analyzed for their history of hormone replacement therapy (HRT). 17 women were excluded from the present study because of data discrepancy. 392 women (67.23%) were non-hormonal users, and 191 women (32.6%) were hormonal users, who had used HRT at least once in their life time. Among the HRT users, 121 women (63.35%) were previous-users and 70 women (36.65%) were current users. Among the 94.24% of the women that use oral hormone therapy, 62.22% are past-users and 37.78% are current-users. The remaining 5.76% administer the hormone through transdermal. Most of the participants used the combined estrogen and progestin treatment. Among these, 80.99% are pastusers and 77.14% are current-users (Table 3).

The current hormone users showed a higher prevalence (10.00%) of high-risk HPV infection, compared to the never-users (5.36%) and the pastusers (4.96%), see Table 3. Among the current users, the women who orally take estrogen had a significant elevated risk of high-risk HPV infection (OR = 9.82; 95% CI 3.02-31.89) when compared to non-hormonal users.

### Discussion

Based on the results from the present study, the authors found that 5.67% of the recruited Thai women had high-risk HPV DNA. The conducted statistical analysis revealed that factors such as smoking, having multiple sexual partners, as well as taking estrogen hormone treatment all contribute to the high-risk HPV infection. The other variable factors, such as oral contraceptive pills usage and the age of having the first sexual intercourse, did not show significant correlation with the high-risk HPV infection. It may be because of the small sample size. In addition, six participants were found to have an abnormal Pap smear, and all of them had high-risk HPV infection. This finding confirmed the previous finding that high-risk HPV infection was highly related to having abnormal cervical cytology(12).

In the present work, the authors used HC2 to detect the high-risk HPV DNA in the participants. HC2 gives positive results when there are more than 5,000 copies of the DNA present, which is at the level that can cause a precancerous lesion on the cervix. Hence,

Characteristics ( $n = 600$ )	HPV infection				Odds	p-value	95% CI
	HPV infected women (n = 34		ed infec en worr		ratio		
Mean age (SD)	53.21	(7.61)	54.46	(7.37)	0.975	0.337	0.926-1.027
Marital status	Number	%	Numbe	er %			
Married	28	84.8	405	80.0	1		
Divorced	2	6.1	39	7.7	0.742	0.691	0.170-3.232
Widowed	3	9.1	62	12.2	0.700	0.567	0.207-2.371
Education							
None educated	2	5.9	11	1.95	3.188	0.154	0.648-15.694
Primary school	11	32.4	166	29.3	1.162	0.714	0.521-2.591
High school	6	17.6	126	22.3	0.835	0.716	0.316-2.203
More than 2 years college	15	44.1	263	46.5	1		
Gynecologic history							
Perimenopause women	13	38.2	138	24.4	1		
Menopausal women age 45-50 years	13	38.2	289	51.0	0.478	0.068	0.216-1.057
Menopausal women age more than 50 years	8	23.5	139	24.6	0.611	0.289	0.246-1.520
Age of first intercourse (years)							
Less than 20 years	10	30.3	136	25.7	1.691	0.299	0.627-4.563
21-25 years	16	48.5	232	43.9	1.586	0.321	0.638-3.943
More than 26 years	7	21.2	161	30.4	1		
Last Sexual intercourse							
Within 1 week	10	30.3	122	23.1	1.778	0.185	0.759-4.166
Within 1 months	10	30.3	125	23.6	1.735	0.204	0.741-4.064
More than 6 months	13	39.4	282	53.3	1		
Parity							
0	5	14.7	92	16.3	1		
1-2	13	38.2	276	48.8	0.867	0.791	0.301-2.497
3 or more	16	47.1	198	35.0	1.487	0.452	0.529-4.182
Number of partners							
1	22	66.7	453	95.4	1		
$\geq 2$	11	33.3	76	87.4	2.980	0.005	1.389-6.395
History of sexual transmitted disease (STD)							
Never	30	88.2	529	93.5	1		
Ever	4	11.8	37	6.5	1.906	0.248	0.638-5.700
Smoking							
Never	29	85.3	542	95.8	1		
Ever but already quit	3	8.8	20	3.5	2.803	0.112	0.788-9.980
Current smoker	2	5.9	4	0.7	9.345	0.012	1.643-53.134
Oral contraceptive pill (OCP)							
Never used	15	44.1	326	57.6	1		
ever used less than 5 years	14	41.2	162	28.6	1.878	0.101	0.885-3.985
ever used more than 5 years	5	14.7	78	13.8	1.393	0.533	0.492-3.949
Condom use				<b>.</b>			
Never used	16	47.1	337	59.5	1	0.007	0.004 1.051
Ever used but not often	11	32.4	118	20.8	1.963	0.097	0.886-4.351
Sometime	5	14.7	79	14.0	1.333	0.586	0.474-3.748
Almost/ every time	2	5.9	32	5.7	1.316	0.722	0.290-5.983

 Table 1. Characteristics and univariate analysis of risk factors associated with high-risk Human Papillomavirus (HPV) infection

Characteristics           Number of partners	HPV infection				Adjusted odds ratio	p-value	95% CI
	HPV infected women $(n = 34)$		Non-HPV infected women $(n = 566)$		ouds futio		
	Number	%	Number	%			
1	22	66.7	453	85.6	1		
$\geq 2$	11	33.3	76	14.4	2.645	0.017	1.188-5.892
Smoking							
Never	29	85.3	542	95.8	1		
Ever but already quit	3	8.8	20	3.5	1.812	0.381	0.479-6.858
Current smoker	2	5.9	4	0.7	7.661	0.025	1.285-45.654

Table 2. Multivariate analysis of risk factors associated with high-risk Human Papillomavirus (HPV) infection

 Table 3. Multivariate risk of high-risk HPV infection and correlation by status of the never, past and current users for hormonal replacement therapy (HRT)

HRT category	n	Results of HPV test		Adjusted OR	95%	p-value	
		HPV+	HPV-	011	Lower	Upper	
Never users	392	21	371	1.000			
Past users	121	6	115	0.922	0.363	2.339	0.864
Oral route	112	5	107	0.826	0.304	2.241	0.707
E only	16	0	16				N/A
E/P	96	5	91	0.971	0.356	2.644	0.954
Transdermal route	9	1	8	2.208	0.264	18.487	0.465
E only	7	1	6	2.944	0.339	25.587	0.328
E/P	2	0	2				N/A
Current users	70	7	63	1.963	0.801	4.809	0.140
Oral route	68	7	61	2.027	0.827	4.973	0.123
E only	14	5	9	9.815	3.021	31.887	< 0.001
E/P	54	2	52	0.679	0.155	2.982	0.609
Transdermal route	2	0	2				N/A
E only	2	0	2				N/A
E/P	0	0	0				N/A

E = estrogen, P = progesterone, N/A = not analysis due to small sample size

the infection occurrence may not be accurate. If, on the other hand, Polymerase Chain Reaction (PCR) technique was used as previously reported<sup>(13)</sup>, the infection occurrence may be higher because PCR detect event one copy of HPV DNA. However, both HC2 and PCR assays have shown results that are compatible to those that are routinely used in the triage of ASCUS cases<sup>(14)</sup>. Moreover, high-risk HPV infection can cause not only cervical cancer but also the other genital cancer<sup>(14,15)</sup>.

The age factor has always been considered as a major contributor to HPV infection. In some

countries, the occurrence of the infection seems to decline with increasing age. However, some studies showed that HPV infection tends to affect the women who are younger than 25 years old, and those that are in between 55-64 years of age<sup>(6,7)</sup>. In the present report, results showed an increase tendency for the women who were older than 45 years old to be prone to the infection. This percentage is comparable to the studies that were conducted previously in Northern and Southern Thailand, as well as in Lumpang and Songkla provinces. Those studies showed that 9.8% of the women who were younger than 35 years old and

4.5-6.0% of the women who were older than 35 had HPV infection<sup>(15)</sup>.

Ishida et al<sup>(16)</sup> and Kanjanavirojkul et al<sup>(17)</sup> found that the smoking history of the partner also contributed to high-risk HPV infection. However, the authors did not study this information. The present finding that the high-risk HPV infection was associated to having multiple sexual partners is consistent with Ishida et al<sup>(16)</sup>. Furthermore, the authors' finding that current- and past- postmenopausal hormonal users did not have an increased risk of the infection, when compared to the hormonal non-users, is consistent with Smith et al<sup>(18)</sup>. However, after a close examination, it was determined that the exposure duration to the combined hormones did increase the risk in the pastusers<sup>(19)</sup>. Although a recent finding from WHI and some studies showed that postmenopausal hormone therapy increased the risk of breast cancer<sup>(20,21)</sup>, the HRT is beneficial in other areas, such as relieving vasomotor symptoms, prevention and treatment of osteoporosis, and improving the quality of life. The authors' finding on the association between the high-risk HPV infection and the estrogen HRT is different from Kedzia et al<sup>(22)</sup>, which showed a significant relationship between level of progesterone in the blood serum and the prevalence of the HPV DNA.

The present study had many limitations. First, the present study was a cross sectional study so the authors could not identify the persistent HPV infected women. Second, the authors relied only on the medical records at the hospital as a source of information. This is only partially efficient, since some of the participants may have received HRT from other hospitals. In addition, the authors did not include duration as one of variables in the present study. Thirdly, the authors were able to recruit only a small number of women who are current HRT users, compared to the past- and the never-users. This also applies to the number of women who administer HRT transdermally, compared to those who take oral HRT. With a larger population size that contains larger numbers of the subgroups, we may be able to obtain information that provides a better understanding of high-risk HPV infection. Finally, further studies using randomized controls will be needed to analyze the correlation between the high-risk HPV infection and the postmenopausal hormonal therapy.

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### ความชุกของการติดเชื้อฮิวแมนแพบพิลโลมาไวรัสชนิดรุนแรง และความสัมพันธ์กับการใช้ การรักษาด้วยฮอร์โมนหลังหมดประจำเดือนในสตรีไทยอายุตั้งแต่ 45 ปีขึ้นไป

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วัตถุประสงค์: เพื่อศึกษาความชุกของการติดเชื้ออิวแมนแพบพิลโลมาไวรัสชนิดรุนแรงและศึกษาความสัมพันธ์ ระหว่างการติดเชื้ออิวแมนแพบพิลโลมาไวรัสชนิดรุนแรงกับการใช้ฮอร์โมนทดแทนในสตรีไทยอายุตั้งแต่ 45 ปีขึ้นไป วัสดุและวิธีการ: การศึกษาแบบตัดขวางศึกษาในสตรีอายุตั้งแต่ 45 ปี ขึ้นไปที่มารับการตรวจที่คลินิกวัยทอง โรงพยาบาลพระมงกุฎเกล้า มีสตรีที่ใช้ฮอร์โมนทดแทนจำนวน 191 คน และไม่ได้ใช้จำนวน 392 คน โดยตรวจหา ดีเอ็นเอของเชื้ออิวแมนแพบพิลโลมาไวรัสด้วยวิธี Hybrid Capture 2 ซึ่งใช้ตัวจับ อาร์เอ็นเอ ของเชื้อชนิดรุนแรง ผลการศึกษา: ความชุกของการติดเชื้ออิวแมนแพบพิลโลมาไวรัสชนิดรุนแรงเท่ากับร้อยละ 5.67 โดยพบการติดเชื้อ ในสตรีจำนวน 34 คนใน 600 คน เมื่อแบ่งตามสถานภาพการใช้ฮอร์โมนพบว่าความชุกของการติดเชื้อนี้เท่ากับร้อยละ 5.36, 4.96 และ 10.00 ในกลุ่มที่ไม่ได้ใช้ฮอร์โมน เคยใช้ฮอร์โมนและกำลังใช้ฮอร์โมนตามลำดับ จากการศึกษาไม่พบ การเพิ่มขึ้นอย่างมีนัยสำคัญของการติดเชื้ออิวแมนแพบพิลโลมาไวรัสชนิดรุนแรงในกลุ่มที่เคยใช้ (adjusted odd ratio (OR) = 0.92, 95% confidence interval (CI) = 0.36-2.34) และกำลังใช้การรักษาด้วยฮอร์โมน (adjusted OR = 1.96, 95% CI = 0.80-4.81) เมื่อเปรียบเทียบกับกลุ่มที่ไม่เคยใช้ อย่างไรก็ตามพบว่าสตรีที่กำลังใช้ฮอร์โมนทดแทน ชนิดเอสโตรเจน อย่างเดียวแบบกินมีความเสี่ยงในการติดเชื้อฮิวแมนแพบพิลโลมาไวรัสชนิดรุนแรงเพิ่มขึ้น อย่างมีนัยสำคัญทางสถิติ (adjusted OR = 9.82, 95% CI = 3.02-31.89)

**สรุป**: ความชุกข<sup>้</sup>องการติดเชื้อฮิ้วแมนแพบพิลโลมาไวรัสชนิดรุนแรงในสตรีไทย<sup>่</sup>อายุตั้งแต่ 45 ปีขึ้นไป เท่ากับร้อยละ 5.67 และการใช้ฮอร์โมนเอสโตรเจนอย่างเดียวแบบกินสัมพันธ์กับการเพิ่มความเสี่ยงของการติดเชื้อฮิวแมนแพบพิลโลมา ไวรัสชนิดรุนแรง อย่างไรก็ตามจำนวนอาสาสมัครที่เข้าร่วมการศึกษา มีจำนวนไม่มากดังนั้นควรมีการศึกษา เพิ่มเติมต่อไป