RESEARCH ARTICLE

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Prevalence of Human Papillomavirus Infection in Nakhon Ratchasima, Northeastern Thailand

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Abstract

Objective: To assess the prevalence of high-risk human papillomavirus (hrHPV) in Nakhon Ratchasima province, Thailand, which might lead to the formulation of effective HPV vaccination strategies in the region. **Methods:** This study analyzed hrHPV DNA test results, liquid-based cytology findings, and available histological data. The data came from women aged 18 to 62 years who underwent cervical cancer screening in six Nakhon Ratchasima districts: Khon Buri, Soeng Sang, Pak Thong Chai, Nong Bun Mak, Wang Nam Khiao, and Mueang Nakhon Ratchasima. The screening occurred between March 2021 and August 2023. We determined the prevalence of hrHPV infection and performed statistical analysis using chi-square tests and multiple logistic regression. **Result:** A total of 16,209 hrHPV DNA test results were included in the analysis, revealing an overall hrHPV prevalence of 8.03% (1,301/16,209). The most frequently detected category was other hrHPV types (5.62%), followed by HPV type 16 (1.78%) and type 18 (0.62%). A single case of co-infection involving HPV type 16 and another hrHPV type was identified. A significant association between age and hrHPV infection was found ($p<0.001$), with the highest prevalence occurring in women aged 30–39 years. Additionally, increasing age was significantly linked to a decline in hrHPV infection rates. **Conclusion:** This study identifies age as a significant risk factor for hrHPV infection. To enhance hrHPV surveillance, we recommend using tests that detect specific HPV types, which will enable a more detailed assessment of prevalence. Furthermore, conducting similar prevalence studies across different regions in Thailand will provide essential data to guide future HPV vaccination policies.

Keywords: Human Papillomavirus Viruses- Uterine Cervical Neoplasms- Early Detection of Cancer- Prevalence Studies

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Introduction

In 1941, Dr. George Nicholas Papanicolaou introduced the conventional Pap test in his research paper "The Diagnostic Value of Vaginal Smears in the Carcinoma of the Uterus", published in the American Journal of Obstetrics and Gynecology[1]. Since then, this test has been widely utilized for cervical cancer screening, playing a significant role in reducing mortality from the disease [1]. In 1996, the U.S. Food and Drug Administration approved liquid-based cytology (LBC) as an alternative to the traditional Pap smear [2]. Compared to the conventional test, LBC provides several benefits, including a clearer background, fewer air-drying artifacts, and a lower rate of unsatisfactory specimens [2-5]. Currently, high-risk human papillomavirus (hrHPV) test is being proposed as a primary screening method due to its higher sensitivity in detecting high-grade squamous intraepithelial lesion compared to cytology alone [6-10]. Infection with hrHPV is the leading cause of cervical cancer, particularly squamous cell carcinoma. The World Health Organization (WHO) designates HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 as high-risk genotypes [11]. These hrHPV strains are associated with over 90–95% of squamous cell carcinoma cases [11], with HPV type 16 alone accounting for approximately 50% of cervical cancers [12]. Collectively, HPV types 16 and 18 contribute to around 66% of cervical cancer cases and 70% of squamous cell carcinomas [11, 12].

As of 2020, the primary hrHPV test has been incorporated into Thailand's national cervical cancer screening program. This screening is available for all women aged 30-59 years. It is also offered to women aged 15-29 years who face a high risk of HPV infection, such as those with an early sexual debut, multiple sexual partners, or a history of unsafe sex. In 2017, the government launched an HPV vaccination program that initially targeted fifth-grade schoolgirls. This program was later expanded in 2023 to include women aged 11–20 years. At present, three types of HPV vaccines are commercially available: a bivalent vaccine (targeting HPV types 16 and 18), a quadrivalent vaccine (covering HPV types 6, 11,

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16, and 18), and a nonavalent vaccine (protecting against HPV types 6, 11, 16, 18, 31, 33, 45, 52, and 58) [13]. This study aimed to evaluate the prevalence of hrHPV in Nakhon Ratchasima province, which may contribute to the development of effective HPV vaccination policies in the region.

Materials and Methods

This cross-sectional analytical study utilized retrospective observational data and adhered to established international guidelines for human research protection, including the Declaration of Helsinki, The Belmont Report, CIOMS Guideline, and the International Conference on Harmonization in Good Clinical Practice (ICH-GCP). Ethical approval was granted by the Human Research Ethics Committee, Suranaree University of Technology, Nakhon Ratchasima, Thailand (EC-66-114). Patient informed consent was waived since all data were sourced from existing records available on the National Cancer Institute website and laboratory information system. The dataset comprised hrHPV DNA test, LBC, and histologic data from specimens processed at Suranaree University of Technology Hospital between March 2021 and August 2023. To maintain patient confidentiality, all identifying information was removed.

This study encompassed all cervical specimen resultes from women who participated in cervical cancer screening and were sent to Suranaree University of Technology Hospital (SUTH) for hrHPV DNA testing between March 2021 and August 2023. These specimens were sourced from subdistrict health-promoting hospitals and district hospitals in Mueang Nakhon Ratchasima, Khon Buri, Soeng Sang, Pak Thong Chai, Nong Bun Mak, and Wang Nam Khiao districts, as well as from Suranaree University of Technology Hospital in Mueang Nakhon Ratchasima district. Collected demographic data included patient age, district, and hospital type.

The cervical specimens were preserved in PreservCyt® Solution vial (Hologic Inc, Massachusetts, USA) and sent to the molecular biology laboratory unit at Suranaree University of Technology Hospital for hrHPV DNA testing. Testing was performed using the Roche Cobas® 4800 system (Roche Diagnostics Ltd., Rotkreuz, Switzerland) via real-time polymerase chain reaction (PCR). The results were categorized as 'Detected" and "Not Detected" for HPV type 16, HPV type 18, and other high-risk HPV types, including 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. If a sample tested positive for other hrHPV types, LBC was performed using the ThinPrep® Processor, model T2000 (Hologic Inc., Massachusetts, USA). Cytotechnologists and board-certified cytopathologists interpreted the LBC slides following the Bethesda System for Reporting Cervical Cytology. Patients with atypical squamous cells of undetermined significance (ASC-US) or more severe findings underwent colposcopy for histological evaluation.

The sample size was calculated using Cochran' s (1977) formula [14] with reference to data from Phetphichai et al., who investigated the prevalence of 14 hrHPV types in Pichit, Uthai Thani, Chai Nat, and Kamphaeng Phet. Their study reported an hrHPV infection prevalence of 7.2% in these provinces [15].

The calculation for the sample size was conducted as follows:

$$n = \frac{z^2_{\alpha}/_2 P(1-P)}{e^2}$$

Therefore, the minimum sample size required for this study was determined to be 53. Data analysis was conducted using IBM® SPSS® Statistics software (version 29, licensed to Suranaree University of Technology). Descriptive statistics, including frequency and percentage, were used to summarize the demographic characteristics of the participants. A significance level of 0.05 (α) was applied, and two-tailed tests were utilized. The Chi-square test was employed to examine the relationship between age group, hospital type, and hrHPV infection, with a p-value of less than 0.05 considered statistically significant. A multiple logistic regression analysis was performed to identify risk factors for hrHPV infection, with factors deemed statistically significant at a p-value of less than 0.05.

Results

A total of 16,210 hrHPV test results were collected, with one result being excluded due to invalidity. The final analysis included 16,209 hrHPV results from women aged 18 to 62. These samples comprised 6,894 samples from Mueang Nakhon Ratchasima, 4,158 from Khon Buri, 1,241 from Soeng Sang, 1,318 from Pak Thong Chai, 1,520 from Nong Bun Mak, and 1,078 from Wang Nam Khiao districts (Table 1). The results consisted of 1,301 cases (8.03%) with any detected hrHPV, 289 cases

Table 1. Demographic Data of 16,209 Women who Received Cervical Cancer Screening by hrHPV DNA Test in This Study

Data	N (%)
Age (years)	
11-20	2 (0.01)
21-30	368 (2.27)
31-40	4,110 (25.36)
41-50	5,844 (36.05)
51-60	5,884 (36.30)
61-70	1 (0.01)
District	
Mueang Nakhon Ratchasima	6,894 (42.53)
Khon Buri	4,158 (25.65)
Soeng Sang	1,241 (7.66)
Pak Thong Chai	1,318 (8.13)
Nong Bun Mak	1,520 (9.38)
Wang Nam Khiao	1,078 (6.65)
Hospital type	
University hospital	6,863 (42.34)
District hospital	1,210 (7.46)
Subdistrict health promoting hospital	8,136 (50.19)

Table 2. Chi Square Analysis for the Association between Age and hrHPV Infection

Factor	hrHPV result		p-value
	Negative	Positive	
Age (years)			<0.001**
18-29	17 (0.1%)	6 (0.5%)	
30-39	3,518 (21.7%)	452 (2.8%)	
40-49	5,237 (32.3%)	443 (2.7%)	
50-59	6,133 (37.8%)	400 (2.5%)	
Hospital type			<0.001**
University hospital	6,159 (38.0%)	704 (4.3%)	
Other types of hospital (district hospital and subdistrict health promoting hospital)	8,749 (54.0%)	597 (3.7%)	

[1]hrHPV, high-risk human papillomavirus; **p<0.01

Table 3. Multiple Logistic Regression Analysis According to Age and Hospital Type

Factor	OR	p-value	95% Confidence Interval	
			Lower	upper
Hospital type	0.681	0.000**	0.604	0.767
Age (years)				
18-29	Reference			
30-39	4.498	0.002**	1.757	11.512
40-49	1.711	0.000**	1.476	1.983
50-59	1.225	0.005**	1.064	1.411

[1]OR, odds ratio; **p<0.01

(1.78%) of HPV type 16, 100 cases (0.62%) of HPV type 18, 911 cases (5.62%) of other detected hrHPVs (type 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68), and 1 case (0.01%) of coinfection with HPV type 16 and other hrHPV. The remaining 14,908 cases (91.97%) did not show any detection of hrHPV (Supplement 1).

The prevalence of any hrHPV was found to be 10.23% in Mueang Nakhon Ratchasima, 6.59% in Khon Buri, 6.61% in Soeng Sang, 6.15% in Pak Thong Chai, 5.66% in Nong Bun Mak, and 6.77% in Wang Nam Khiao districts (Supplement 2). Regarding hospital type, the prevalence of any hrHPV was 10.26% in the university hospital, 8.10% in district hospitals, and 6.13% in subdistrict health-promoting hospitals. In all districts and hospital types, the proportion of other hrHPV infections was higher than that of HPV types 16 and 18, respectively.

The cytology result for the single co-infection case (0.01%) with HPV type 16 and another hrHPV was negative for intraepithelial lesion or malignancy (NILM). Among the other hrHPV cases, the cytology results were as follows: 1 case (0.11%) was unsatisfactory for evaluation, 634 cases (69.52%) of NILM, 89 cases (9.76%) of atypical squamous cells of undetermined significance (ASC-US), 21 cases (2.30%) of atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion (ASC-H), 108 cases (11.84%) of low-grade squamous intraepithelial lesion (LSIL), 48 cases (5.26%) of high-grade squamous intraepithelial lesion (HSIL), 2 cases (0.22%) of HSIL with features suspicious for invasion, 4 cases (0.44%) of atypical endocervical cells, not otherwise specified (NOS), 1 case (0.11%) of atypical endometrial cells, NOS, 1 case (0.11%) of atypical endometrial cells, favor neoplastic, and 2 cases (0.22%) of atypical glandular cells, NOS (Supplement 3).

Histological results were obtained for 43 cases with other hrHPV infections, which included 4 cases of ASC-US, 7 cases of ASC-H, 9 cases of LSIL, 18 cases of HSIL, 3 cases of atypical endocervical cells NOS, 1 case of atypical endometrial cells favoring neoplasia, and 1 unsatisfactory case. No squamous intraepithelial lesion was identified in any ASC-US cases. Among the cases, one ASC-H, two LSIL, and one atypical endocervical cells, NOS revealed HSIL on histological evaluation. In addition, one case with cytologic HSIL was found to have keratinizing squamous cell carcinoma with HSIL in the histological specimen (Supplement 4).

Chi-square analysis revealed significant associations between hrHPV infection and both age group and healthcare hospital type (Table 2). However, the hospital type may not have clinical relevance in terms of hrHPV infection.

In the multiple logistic regression analysis, age was considered as a variable and hospital type as a covariate. Odds ratios (OR) and 95% confidence intervals (95% CI) were adjusted for hospital type. The analysis indicated that age was a risk factor for hrHPV infection. In the study, there was also a tendency for a decrease in hrHPV infection with increasing age. The population was categorized into four age groups. The prevalence of hrHPV was 0.5% in women aged 18-29 years, 34.7% in women aged 30-39 years, 34.1% in women aged 40-49 years, and 30.7% in women aged 50-59 years. Women aged 18–29 years were used as the reference group. The results

showed that the risk of hrHPV infection was significantly higher in women aged 30–39 years (OR = 4.49 (95% CI 1.757–11.512), p = 0.002), 40–49 years (OR = 1.71 (95% CI 1.476–1.983), p < 0.001), and 50–59 years (OR = 1.225 (95% CI 1.064–1.411), p = 0.005) (Table 3).

Discussion

Numerous studies have indicated that the prevalence of hrHPV among Thai female population ranges from 3.4-14% [16-22]. In other Asian populations, the prevalence of hrHPV has been reported as 4.53% in Malaysia [23], 11-20.45% in China [24-27], 23.06% in Iran [28], and 33.7% in South Korea [29]. The 8.03% hrHPV prevalence found in our study is within the range observed in both Thai and other Asian populations.

In our study, the prevalence of other hrHPV types (types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) was the highest, followed by types 16 and 18, respectively. Chaopotong et al., reported in their study of HPV infection in Thai women aged 25-65 years, found that HPV group B (types 35, 39, 51, 56, 59, 66, and 68) was the most common, followed by type 16, HPV group A (types 31, 33, 52, and 58), and type 18 [20]. Sangrajrang et al. [17] in their study on the accuracy of Pap smear and HPV screening in Thai women aged 30-60 years, also found that other hrHPV types were the most common, followed by types 16 and 18. Vongpunsawad et al., in their study of HPV infection among Thai schoolgirls aged 10-18 years, reported that type 16 was the most prevalent, followed by types 58, 51, 52, and 66 [30]. Tangjitgamol et al., in their study of hrHPV infection prevalence among Bangkok women aged 25-65 years, revealed that type 16 was the most common, followed by types 52, 58, 18, and 51[19]. The strength of our study is that it involved the largest population compared to previous studies conducted in Thailand. The most common type of hrHPV identified in our study was consistent with findings from earlier researches in the Thai population [17, 20]. This suggested that other hrHPV types remain a major public health concern in Thailand. From a clinical perspective, the bivalent vaccine, which is less expensive than the nonavalent vaccine, may not provide sufficient protection for the Thai population. A cost-benefit analysis is needed to evaluate the HPV vaccination policy from a health economics aspect.

Kantathavorn et al., in their study on genotyping of HPV infection in teaching hospital and community settings, found that the most common hrHPV types were 52, 16, 51, 58, 39, and 18 in the teaching hospital and 16, 51, 52, 58, 18, and 59 in community setting[31]. In our study, both district and subdistrict health-promoting hospitals represent the population in their community, while SUTH serves as a teaching hospital. Thus, our results showed that the group of hrHPV types other than 16 and 18 was more prevalent than types 16 and 18 in both teaching and community-based hospitals, which differs from the findings of Kantathavorn et al. However, a limitation of our study is the categorization of hrHPV DNA results into broad groups: type 16, type 18, and other hrHPV. If we had further subclassified the "other hrHPV" category into specific types, it is possible that type 16 might have emerged as the most common hrHPV type in our study. Therefore, hrHPV tests capable of reporting detections by specific types are recommended to more accurately determine the prevalence of each hrHPV type. A better understanding of the distribution of hrHPV types in different regions could inform HPV vaccination policy development.

The highest prevalence of hrHPV infection in our study was observed in women aged 30-39 years, which is consistent with findings from a previous study in the Thai population[18]. The peak age of infection in this study was slightly higher than reported in earlier research, but the trend of decreasing infection rates with increasing age remained consistent [31]. This could be attributed to higher sexual activity among younger women, combined with the fact that nearly 90% of HPV infections are eradicated by the immune system within two years, which may explain the infection trend observed in our study [32-34]. Immunity from prior HPV infections in older women may also protect them from reinfection [32]. Marks et al. [22] also reported a peak of hrHPV infection in Thai women. The highest prevalence of hrHPV infection among women aged 30-39 years in our study supports the idea that the current hrHPV DNA screening program for all women aged 30-59 years is appropriate. However, the relatively small number of women aged 18-29 years in our study compared to other age groups might have impacted the findings. Future studies should include a larger sample from this age group to provide a more accurate evaluation of hrHPV infection prevalence.

Previous studies have identified several risk factors for HPV infection in women, including age [16, 19, 35], premenstrual status [19], education [35], employment status [28], marital status [28], prior sexual activity [19, 36], age at first sexual activity [19, 35], timing of recent new sexual partners[36], duration with a regular partner[36], increased number of sexual partners [36, 16, 19, 22, 28, 35, 37], their male sexual partners having other partners [36, 22], older male partners [36], younger age at first pregnancy [37], gravida status [28], increased parity [37], breastfeeding status [28], long-term oral contraceptive usage [36, 22, 37], non-condom contraception [35], Chlamydia infection [36, 22, 37], Trichomonas infection [37], bacterial vaginosis [22, 37], decreased Lactobacillus in vagina [37], smoking [28, 37], human immunodeficiency virus (HIV) infection [37], use of immunosuppressive medication in inflammatory bowel disease (IBD) patients [37], decreased vegetable consumption [37], and increased vitamin C or selenium intake [37]. On the contrary, a previous study in Thai women found that factors such as age, education level, marital status, age ≤ 20 years at first sexual activity, and increased number of sexual partners were not associated with HPV infection [38]. In our study, age was identified as a risk factor for hrHPV infection, consistent with findings from some earlier studies in the Thai population [16, 19]. A limitation of the retrospective data in our study was the limited demographic data collected, which included only age, district, and hospital type. In our future cohort study, more comprehensive data on the risk factors

is needed to better understand hrHPV infection in our population. These findings might be useful to improve prevention measures for hrHPV infection in this region.

In conclusion, this study reported the prevalence and most common types of hrHPV infection in Nakhon Ratchasima, Thailand, and identified age as a significant risk factor for hrHPV infection. However, the results may not fully reflect the prevalence, most common types, or risk factors for hrHPV infection in other populations in Northeastern Thailand or the country as a whole. We recommend the use of hrHPV tests that provide specific type detection to more accurately determine the prevalence of each type. Furthermore, conducting prevalence across various regions in Thailand will yield valuable insights for the development of future HPV vaccination policies.

Author Contribution Statement

Conceptualization: NP. Data curation: NP. Formal analysis: ST. Funding acquisition: NP. Methodology: NP, ST. Project administration: NP. Visualization: NP. Writing - original draft: NP. Writing - review & editing: NP, ST.

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Scientific Body Approval

The research project was approved by the Department of Clinical Research, Suranaree University of Technology Hospital and the Human Research Ethics Committee, Suranaree University of Technology, Nakhon Ratchasima, Thailand. This research is not a part of an approved student thesis.

Ethical Declaration

The study was approved by the Human Research Ethics Committee, Suranaree University of Technology, Nakhon Ratchasima, Thailand (EC-66-114).

Availability of data

The data that support the findings of this study are available from the corresponding author, NP, upon reasonable request.

Conflict of Interest

The authors have no conflicts of interest associated with the material presented in this paper.

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