RESEARCH COMMUNICATION

Detection of Human Papillomavirus in Intraepithelial Lesions and Carcinoma of the Cervix Uteri in Southern Thai Women

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Abstract

    <u>Objective</u>: To evaluate the prevalence of high-risk type human papillomavirus (HR-HPV) in preneoplastic lesions and invasive squamous cell carcinoma (SCC) of the cervix uteri in southern Thai women. <u>Materials and Methods</u>: A total of 148 formalin-fixed, paraffin-embedded blocks of cervix tissue were retrieved from the files of the Department of Pathology, Prince of Songkla University Hospital. They were classified as negative for intraepithelial lesion (NIL) in 37 cases, low grade lesion (LGL) in 58 cases, high grade lesion (HGL) in 39 cases and SCC in 14 cases. HR-HPV DNA was tested with an Amplicor HPV ® (Roche Diagnostics) detection kit. <u>Results</u>: Of the 111 cases, 42 of 58 LGLs (72.4%), 34 of 39 HGLs (87.2%) and 13 of 14 SCCs (92.9%) were positive for HR-HPV DNA. In 37 cases of histologically normal cervix, there were 15 cases that showed the presence of HR-HPV DNA. Applying the HR-HPV results for NILs to the general population, the age standardized incidence rate of HR-HPV infection in the normal Thai population was 12.8%. <u>Conclusion</u>: HR-HPV DNA can be found in all grades of intraepithelial lesions and carcinoma of the cervix uteri, even in the histologically "normal" looking cervix. These results provide strong evidence for a role in carcinogenesis of the cervix uteri and the existence of a non-productive or latent period of HPV infection.

Key Words: Human papillomavirus - HPV - cervical cancer

Introduction

    Carcinoma of the cervix uteri is the most common malignant tumor among Thai women with an age-standardized incidence rate (ASR) of 19.5 for Thailand (Pengsaa and Jindawijak, 2003). The incidence and the number of new cases of cervical cancer are increasing, with over 8,000 new cases estimated in Thailand in 2008 (Sriplung, 2003).

    The oncogenic or high-risk human papillomavirus (HR-HPV) is notorious as the main cause of preexisting non-invasive squamous intraepithelial lesions and carcinoma of the cervix (Baseman and Koutsky, 2005; Steenberger et al., 2005). The well-known mechanism of HR-HPV-caused intraepithelial lesions or carcinoma is the integration of the viral genome into the host cell DNA at the basal cell layer. The E6 and E7 of the HR-HPV interfere with cell cycle control and apoptosis via interaction with the p53 and RB genes respectively. This integration and over-expression of E6/E7 result in cell transformation that, in combination with alteration of other host genes, can persist or progress into a preneoplastic or malignant stage (McGlennen, 2000; Steenberger et al., 2005).

    The prevalence of HPV in cervical intraepithelial lesions and carcinoma has been extensively studied. Bosch and de

Sanjosé reported a 15.1% median HR-HPV prevalence among all women worldwide (ranging from 2% in North Vietnam to 42.8% in Zimbabwe) (2003). Muñoz et al, from the International Agency for Research on Cancer (IARC) multicenter cervical cancer study group, pooled and analyzed 11 case-control data studies regarding HPV in squamous cell carcinoma (SCC) in 9 countries, including Thailand. Their results showed HPV DNA detection in 90.7% of patients with cervical carcinoma and 13.4% in the controls. The pooled odds ratio for cervical carcinoma associated with any HPV was 158.2 (95% CI 113.4-220.6) (Muñoz et al., 2003). A meta-analysis by Clifford et al revealed an overall detection of HPV DNA of 83-89% in invasive cervical cancer and, if considering only SCC, the prevalence of HPV was 87.3% (2003).

    Regarding HPV data in Thailand, the percentages of HPV DNA in cervical lesions varied according to type of specimen, probe and technique used in detection, but overall HPV DNA was demonstrated in 39% (Siritanikorn et al., 1997) to 100% (Limpaiboon et al., 2000) of SCC cases. The lower prevalence possibly resulted from a limited number of HR-HPV type-specific probes used and the in situ hybridization technique (Siritanikorn et al., 1997) which is less sensitive than the polymerase chain reaction (PCR) and dot blot hybridization methods used in later studies

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(Bhattarakosol et al., 1996; 2002; Chichareon et al., 1998; Lertworapreecha et al., 1998; Limpaiboon et al., 2000; Thomas et al., 2001). In premalignant lesions, HPV DNA was found in 33% of low-grade lesions (LGL) (Bhattarakosol et al., 2002) and 38% to 75% of high-grade lesions (HGL) (Bhattarakosol et al., 2002; Lertworapreecha et al., 1998; Siritanikorn et al., 1997; Thomas et al., 2001). The overall HPV prevalence in Thai women ranged from 2.7 to 15.7%. So far, most of these HPV studies in Thai women have been from the central region of Thailand, with only 2 studies from Songkhla province, in the southern part of Thailand. One study examined exfoliated cervical cells from a normal population (Sukvirach et al., 2003) and the other (Chichareon et al., 1998) looked at case-control studies in fresh tissue from only cervical carcinoma. Our study aimed to evaluate the prevalence of HR-HPV in formalin-fixed, paraffin-embedded tissue from normal, preneoplastic lesions and SCC of the cervix uteri in southern Thai women.

Materials and Methods

Case selection

The study was approved by the Institutional Review Board and Ethics Committee of the Faculty of Medicine, Prince of Songkla University. Pathological glass slides from 2000-2004 inclusive of cervical biopsies and/or hysterectomies diagnosed as koilocyte, mild dysplasia, moderate dysplasia, severe dysplasia, in situ squamous cell carcinoma and invasive squamous cell carcinoma were retrieved from the records of the Department of Pathology, Songklanagarind hospital, Prince of Songkla University. All cases were reviewed and one paraffin block from a representative slide was selected in each case.

HPV DNA detection

Each paraffin block was cut into 10, 10-micron thick sections. The unstained sections were mapped with H&E slide, and then the representative areas were manually scraped, case by case, with a sterile blade and collected into sterile microtubes. DNA was extracted using the QAIamp DNA Mini Kit®(Qaigen) according to the instructions of the manufacturer. The HPV DNA was assayed using the Amplicor HPV test® (Roche Diagnostic), which is a PCR-based technique. In brief, the working master mix that contained primer pairs for DNA from 13 HR- HPV genotypes (HPV-16,-18,-31,-33,-35,-39,-45,-51,-52,-56,-58,-59, and -68) and β-globin DNA was added to each reaction tube. These tubes were placed in a Thermal Cycler (GeneAmp PCR System 9700™, Roche Diagnostics) and the program was initiated as recommended by the manufacturer. After amplification, denaturation solution was added to chemically denature the HPV amplicon and the β-globin amplicon into single-stranded DNA. The HPV HYB solution and the denatured amplicon were added to separate wells of the microwell plates (MWP) coated with either HPV high-risk probes or β-globin specific oligonucleotide probes, and then mixed, covered and incubated at 37˚C for 1 hour.

After that the AV-HRP solution was added to each well and incubated at 37˚C for 15 minutes. Then a working substrate was added to each well and they were placed in a dark place at room temperature for 10 minutes to allow color development. After that, STOP solution was added to each well and the wells were then measured at A_{450} within 10 minutes.

The interpretation was positive with a HPV result of $A_{450} \geq 0.20$ and as negative with HPV of $A_{450} < 0.20$ and the β-globin was positive with $A_{450} \geq 0.20$. If both results were <0.20, the test was considered as invalid and was repeated. Positive and negative control cases were provided by the manufacturer and run concurrently with each PCR test.

Statistical analysis

The program R (version 2.2.0) was used for data manipulation. The age-standardized prevalence of HPV DNA detection was computed using the world standard population as a reference (R Development Core Team, 2005).

Results

There were 148 cases included in this study (Table 1). Fifty-eight cases of koilocyte and/or mild dysplasia were grouped as LGL, 39 cases of moderate to severe dysplasia and in situ squamous cell carcinoma were grouped as HGL and 14 cases were SCC. The age ranged from 24 to 80 years old with a mean age of 43 years. The normal looking cervices (n = 37) were retrieved from hysterectomy specimens which had been tested for indications other than cervical lesions. The mean age of the normal group was 44.7 and ranged from 20 to 64 years old.

HR-HPV DNA was demonstrated in all degrees of cervical intraepithelial lesions with odd ratio 5.85 (95% CI 2.46-14.37). Thirteen out of 14 (92.9%) SCCs contained HR-HPV DNA. The HGL and LGL revealed HR-HPV DNA in 34 out of 39 cases (87.2%), and 42 out of 58 cases (72.4%), respectively. The odds ratio also increased form 3.79 in LGL to 12.34 in SCC.

Fifteen out of 37 normal looking cervices (40%) also showed HR-HPV DNA. Because this group was selected from the hospital-based population, an age-adjustment was performed to match the general population, following which the age-standardized prevalence rate (ASR) of HR-HPV DNA among our general population was 12.75%.

Table 1. Detection of HR-HPV DNA in Cervix Uteri

	HR-HPV+/cases	Odds ratio	95% CI	P value
NIL	15/37	Ref.		
Abnormal	89/111	5.85	2.46-14.37	<0.001
- LGL	42/58	3.79	1.48-10.11	0.003
- HGL	35/39	12.34	3.41-57.89	<0.001
- SCC	13/14	18.10	2.28-842.2	0.001

HR-HPV = high risk type human papillomavirus, NIL = negative for intraepithelial lesion, LGL = low grade lesion, HGL = high grade lesion, SCC = squamous cell carcinoma

Discussion

Carcinoma of the cervix is the most common malignant tumor in Thai women (Pengsaa and Jindawijak, 2003; Sriplung, 2003). High-risk human papillomavirus can be detected in most precancerous lesions and almost all cervical carcinomas. Hence, HR-HPV is accepted as a major carcinogenic pathway of the cervix uteri (Baseman and Koutsky, 2005; Bosch and de Sanjosé, 2003; Clifford et al., 2003; McGlennen, 2000; Munõz et al., 2003; Pengsaa and Jindawijak, 2003).

The overall HPV prevalence in invasive squamous cell carcinoma, according to a meta-analysis of HPV types in cervical cancers worldwide, is 87.3% (ranging from 83% to 89%) (Clifford et al., 2003). In Thailand, the prevalence of HR-HPV in squamous cell carcinoma varies from 39% to 100% according to type of specimen and technique used (Bhattarakosol et al., 1996; Chichareon et al., 1998; Limpaiboon et al., 2000; Siritantikorn et al., 1997). The lowest prevalence is from cervical biopsies using the in situ hybridization technique which is an insensitive test when compared to the PCR technique (Siritantikorn et al., 1997). A previous study from our institute, reported by Chichareon et al (1998), disclosed HR-HPV DNA in 96.5% of SCC cases. They used fresh specimens sent to the IARC laboratory to detect HR-HPV by PCR-based assay. Our study was done in formalin-fixed, paraffin-embedded tissue and used a commercial PCR test kit that included all 13 HR-HPV types. Our results are comparable to Chichareon et al's, even though we used a different specimen type (formalin-fixed, paraffin-embedded tissue vs fresh tissue). HR-HPV DNA was demonstrated in 13 out of 14 cases (92.9%) of invasive squamous cell carcinoma. We found no significant difference in the prevalence of HR-HPV in invasive cervical carcinoma during the past ten years compared to Chichareon et al's. The only negative HR-HPV SCC case in our study showed β-globin at the lower limit level (0.20), even though we repeated the test twice. This negative result may be due to too small an amount of HPV DNA in the specimen or may represent a true HR-HPV-negative cervical cancer.

In premalignant lesions, our data show HR-HPV DNA at 72.4% in LGL and 87.2% in HGL. This result is higher than in previous reports from Thailand. Siritantikorn et al. (1997) studied cervical intraepithelial neoplasia 3 (CIN 3, encompassing HGL) using in situ hybridization. Their results revealed only 38% of CIN3s were positive for HR-HPV DNA. Thomas et al (2001) reported 57% positive HR-HPV in cervicovaginal lavage diagnosed as squamous intraepithelial lesion (SIL) detected by PCR-based technique. Lertworapreecha et al (1998) used the PCR dot blot technique and found HR-HPV DNA in 74% of CIN3s. Bhattarakosol et al (2002) also used a PCR dot blot assay and demonstrated HR-HPV DNA in 75% of CIN3s and only 33.3% of CIN1s (encompassing LGL). The high prevalence of HR-HPV DNA in LSIL in our study should be noted as a caution that the presence of HR-HPV DNA does not always mean a high grade lesion. There are other molecular mechanisms that can be involved in turning a low-grade lesion to a high-grade lesion.

In the general population of Thailand, Sukvirach et al (2003) studied exfoliative cervical cells with PCR Southern Blot analysis among women in Lampang and Songkhla provinces and reported an overall HPV prevalence of 6.3%. The HPV prevalence was higher in Lampang than in Songkhla (9.1% vs 3.9%, respectively). Our data demonstrated HR-HPV DNA in 15 out of 37 "normal looking" cervixes which were resected due to causes other than cervical lesions. This prevalence was high, but the specimens came from a hospital-based population. To apply our data to the general population, we adjusted to the age-standardized prevalence rate (ASR), resulting in an ASR of HR-HPV prevalence 12.8%. This prevalence also shows no significance difference when compared to the study of Chichareon et al (1998), although it is higher than previous reports by the groups of Lertworapreecha et al (1998), Bhattarakosol et al (2002), and Sukvirach et al (2003) (6%, 2.7 % and 6.3% respectively). The possible causes of these different results include the population selected (that is, whether population-based or hospital-based), the area of the study, time of study, type of specimen and technique used. Thus, the morphology alone cannot predict the HPV status. The detection of HR-HPV DNA in normal looking cervices confirmed the existence of the non-productive or latent phase of the HPV infection.

In conclusion, HR-HPV DNA can be detected in the intraepithelial lesions and carcinomas of the cervix uteri. Even in "normal" looking cervixes HR-HPV DNA can be demonstrated. Our findings confirm HR-HPVs' role in carcinogenesis of the cervix uteri and the existence of a latent period of HPV infection. Other steps of molecular pathways that change in consequence of HR-HPV infection and trigger a more precise progression into more severe lesions are under investigation.

Acknowledgements

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