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Prevalence and distribution of HPV type in King Chulalongkorn Memorial Hospital

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- Objective** : *To investigate the prevalence and distribution of human papillomavirus (HPV) type in specimens collecting at King Chulalongkorn Memorial Hospital*
- Design** : *Cross-sectional descriptive study*
- Setting** : *Virology Laboratory Unit, Department of Microbiology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand*
- Subjects** : *All 139 clinical samples were sent to Virology Laboratory Unit, from October 2006 to May 2007*
- Methods** : *All 139 samples were primarily screen positive for the presence of HPV-DNA by Amplicore HPV amplification kit (Roche, USA). They were then continued for typing using Linear Array HPV genotyping kit purchased from Roche.*
- Results** : *Only 123 samples were from women. Sixty-one samples (49.59 %) were single infection with 47 (77.05 %) were high-risk type and 14 (22.95 %) were low-risk type. Another 62 (50.41 %) samples were mixed infection of two types (33; 53.23 %), three types (19; 30.64 %) and equal or grater than four types (10; 16.13 %). Among these types, HPV -52 which belongs to high- risk types were of the highest prevalence (31; 25.20 %) followed by type 16 (30; 24.39 %)*

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Conclusion : *Although the data obtained from laboratory investigation might not be a good representative the figure of the prevalence of the population, at least it is useful for estimating the distribution of HPV type of Thai women. Our results indicated that most Thai women were infected with high-risk types of HPV especially HPV-52 and -16.*

Keywords : *Prevalence, HPV type, King Chulalongkorn Memorial Hospital.*

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- วัตถุประสงค์** : เพื่อสำรวจความชุกและการกระจายของ HPV ไวรัสแบบปิโลมาในโรงพยาบาลจุฬาลงกรณ์
- รูปแบบการวิจัย** : แบบพรรณนาชนิดตัดขวาง ณ จุดเวลาใดเวลาหนึ่ง
- สถานที่ทำการศึกษา** : ห้องปฏิบัติการไวรัสวิทยา ภาควิชาจุลชีววิทยา คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย กรุงเทพมหานคร 10330, ประเทศไทย
- ประชากรที่ศึกษา** : ตัวอย่างจำนวน 139 ตัวอย่างที่ส่งตรวจที่ห้องปฏิบัติการไวรัสวิทยา ตั้งแต่เดือนตุลาคม พ.ศ.2549 ถึง พฤษภาคม พ.ศ. 2550
- วิธีการศึกษา-วัดผล** : ตัวอย่างทั้งหมด 139 ตัวอย่าง ตรวจพบ HPV-DNA แล้วด้วยชุดน้ำยาสำเร็จรูป AmpliCore HPV amplification ทำการตรวจจำแนก HPV ด้วยชุดน้ำยาสำเร็จรูป Linear Array HPV genotyping kit จากบริษัท โรช จำกัด
- ผลการศึกษา** : มีตัวอย่างเพียง 123 ตัวอย่างเท่านั้นที่มาจากผู้หญิง 61 ตัวอย่าง (ร้อยละ 49.59) ตรวจพบเพียง HPV เดียว ซึ่งแยกได้เป็นกลุ่มที่เป็น HPV ความเสี่ยงสูงจำนวน 47 ตัวอย่าง (ร้อยละ 77.05) และ 14 ตัวอย่างร้อยละ 22.95 เป็น HPV อยู่ในกลุ่มความเสี่ยงต่ำ ที่เหลืออีก 62 ตัวอย่าง (ร้อยละ 50.41) เป็นการติดเชื้อที่ผสมโดยพบติดเชื้อสอง HPV (33 ตัวอย่าง; ร้อยละ 53.23) ติดเชื้อสาม HPV (19 ตัวอย่าง; ร้อยละ 30.64) และเท่ากับหรือมากกว่าสี่ HPV (10 ตัวอย่าง; ร้อยละ 16.13) ในจำนวนทั้งหมดพบ HPV HPV 52 มีความชุกสูงสุด คือ 31 ตัวอย่าง (ร้อยละ 25.20) รองลงมาคือ HPV 16 จำนวน 30 ตัวอย่าง (ร้อยละ 24.39)
- วิจารณ์และสรุปผล** : แม้ว่าข้อมูลที่น่ามาวิเคราะห์เป็นข้อมูลที่ได้จากการตรวจทางห้องปฏิบัติการซึ่งอาจเป็นตัวแทนของประชากรได้ไม่มากนัก อย่างไรก็ตามข้อมูลดังกล่าวก็สามารถประมาณการกระจายของ HPV ไวรัสแบบปิโลมาในหญิงไทยได้ ผลการวิเคราะห์นี้แสดงให้เห็นว่าผู้หญิงไทยส่วนใหญ่มีการติดเชื้อ HPV ไวรัสแบบปิโลมาในกลุ่มที่มีความเสี่ยงสูงโดยเฉพาะ HPV 52 และ 16
- คำสำคัญ** : ความชุก, HPV ไวรัสแบบปิโลมา, โรงพยาบาลจุฬาลงกรณ์

Human papillomaviruses (HPVs) are the group of DNA viruses that belong to the family *papillomaviridae*. This virus is the major cause of cervical neoplasia and cervical carcinoma which is the second most common cancer in women worldwide especially in developing countries including Thailand. ⁽¹⁾ Up to date, more than 150 different of HPV types have been recognized ^(2,3) and approximately 40 types are typically transmitted through sexual contact and infect anogenital tract. ⁽⁴⁾ These sexually transmitted HPV types can be separated into two groups corresponding to the capability in developing cervical cancer, i.e., low-risk group such as HPV- 6, 11, 40, 42, 43, 54, 61, 70, 72 and 81 and high-risk group such as HPV- 16,18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82. ⁽⁵⁾ The prevalence and distribution of HPV type is very important data for prevention strategies of HPV infection and cervical cancer. Cervical cancer screening by HPV detection and genotyping have been proved effective in reducing the rates of cervical cancer ⁽⁶⁾ and also screening for HPV genotype distribution are essential for evaluation of the impact of HPV vaccine in each geographic region. The prevalence and distribution profiles of this virus have been observed and found different genotypes in different geographic areas; for example, a study in Korea women found that the most prevalent type in cervical intraepithelial lesion is HPV 16 and 58 whereas the most prevalent type in Germany are HPV 16, 31, 52, 51, 18 and 45. ^(7,8) In Thailand the studies of CIN I-III and invasive cancer in Thai patients revealed that the most prevalent type is HPV 16 (44 %) followed by HPV 18 (16 %). ⁽⁹⁾ However these studies focused on the CIN and invasive cancer patients. Therefore, the data of HPV genotype only represented cancer

lesions of the patients. Hence, in order to observe the prevalence and distribution of HPV infection in the general population, we attempted to explore this information from data obtained in Virology Laboratory Unit.

Materials and Methods

Clinical samples

A total number of 139 HPV screened-positive samples were sent to Virology Laboratory Unit, Department of Microbiology, Faculty of Medicine, Chulalongkorn University during October, 2006 to May, 2007. Among these, 123 samples were from women.

DNA extraction and amplification

All samples were extracted for DNA by using AmpiLute Liquid Media extraction Kit (Roche, USA) and amplified for HPV target by using AMPLICOR HPV Amplification kit (Roche, USA). The procedures of both techniques were based on the company's manuscripts.

HPV-DNA testing

The samples that were positive for HPV-DNA by screening method were further confirmed HPV genotype by using HPV genotyping kit (Linear Array HPV Genotyping test; Roche). This kit is able to detect at least 37 genotypes including 13 high risk HPVs (16,18, 31,33, 35, 39, 45,51,52, 56, 58, 59, and 68). The principle of this test kit is based on the PCR technique amplifying the L1 region of HPV and hybridization technique between amplified products and linear probes that were fixed on the test strip. The procedure of test kit was performed according to recommendation of the company.

Results

In total, 139 samples were screened positive. Only 123 samples were from women. Therefore, these 123 samples were confirmed and typed by HPV genotyping kit (Roche, USA). In this study we found that about half of them (61 samples, 49.59 %) were single infection and another half (62, 50.41 %) were mixed infection of HPV genotype. The mixed infections were composed of 2 (33/123, 26.83 %), 3 (19/123, 15.45 %), 4 and more than 4 types (10/123, 8.13 %). Among the single infection, 47 (77.05 %) samples were classified as high-risk type. The rest 14 samples (22.95 %) were in low-risk type. The most prevalent of high-risk HPV group that were identified in this study was HPV-52 (31/123, 25.20 %) and HPV-16 (30/123, 24.39 %) following by HPV-51 (16/123, 13.01%), HPV-58 (10/123, 8.13%), HPV-39 (10/123, 8.13 %), HPV-18 (9/123, 7.32 %), HPV-56 (9/123, 7.32 %), HPV-68 (8/123, 6.50 %), HPV-59 (7/123, 5.70 %), HPV-33 (4/123, 3.25 %), HPV-31 (2/123, 1.63 %), and HPV-45 (2/123, 1.63 %). A total of 30 HPV-16 positive samples, 11 samples were single infections, 8 samples were double infections and 11 samples were multiple infections. In case of HPV-52, 16 samples were single infections, 7 samples were double infections and 8 samples were multiple infections. The results of HPV genotypes are showed in table 1. Our data showed that mixed infections between high-risk type and low-risk type commonly occurred.

Discussion

Cervical cancer is a serious problem for woman worldwide. Several epidemiology and molecular virology indicated that HPVs are the most significantly associated with this cancer.^(7,10,11) Up to date, there are several types of HPV that are involved in cervical cancer and the epidemiology observation revealed that the prevalence of HPV infection varies

depending on geographic distributions. For example, the prevalence study by Bosch *et al* in 1995 revealed that type 45 predominates in West Africa while the Central and South Americas show comparatively higher frequencies of type 39 and 54. However, the most commonly prevalence HPV found in cervical cancers worldwide are HPV types 16, 18, 33, and 45.⁽¹²⁾ In this present data, the most prevalence types of high-risk HPV are HPV-52, 16, 51, 58, 39, 18, 56, 68, 59, 33, 31 and 45 respectively. This result indicated that the distribution of HPV types in Thai population may be different from CIN and cancer patients. A previous observation indicated that the most prevalence of HPV in CIN and cervical cancer in Thai women were HPV 16, 18 and 33.^(9,13) The possible causes of different results include the technique used for detection and typing. In this study we used commercial kit that is able to detect more HPV types than those previous studies (HPV 6, 11, 16, 18, 33).^(9,13) The different in studied population and types of specimens used in the study are also different. In addition, we demonstrate a high prevalence and high rate of mixed infection of HPV which might imply that there are several types distribute among Thai women and condition of mixed infection may increase the risk of developing cervical cancer. We would like to suggest that HPV screening strategy is probably important for patient care in order to prevent cancer in future. Knowledge of distribution data of HPV type also is important for vaccine strategy in Thai population. Detection of high risk HPV type indicated high possibility of the existence of the non-productive stage or latent infection in the population.

In conclusion, we hereby show the prevalence and distribution of HPV type in Thai population. The result shows a high prevalence and high mixed infection of HPV genotype. Detection and typing of HPV may play important roles in taking care of high-risk women.

Table 1. Distribution of HPV genotype in clinical specimens (Underline = Low-risk type).

Single infection		Double infection		Triple infection		Multiple infection	
HPV type	No	HPV type	No	HPV type	No	HPV type	No
16	11	16/51	1	16/18/58	1	16/18/59/ <u>26</u>	1
18	1	16/52	1	16/51/ <u>6</u>	1	16/35/ <u>42/62</u>	1
33	1	16/58	1	16/58/ <u>11</u>	1	18/52/56/ <u>6</u>	1
39	2	16/18	2	16/51/ <u>81</u>	1	18/ <u>42/54/66</u>	1
45	1	16/35	1	16/56/ <u>83</u>	1	51/52/ <u>40/41</u>	1
51	1	16/59	1	16/ <u>42/84</u>	1	51/ <u>54/61/66</u>	1
52	16	16/ <u>61</u>	1	16/51/ <u>70</u>	1	68/ <u>11/40/54</u>	1
56	4	18/ <u>61</u>	1	16/ <u>53/84</u>	1	33/52/ <u>61/62/71</u>	1
58	6	18/39	1	31/ <u>54/62</u>	1	16/ <u>55/62/72/83</u>	1
68	4	31/68	1	33/51/ <u>84</u>	1	39/52/ <u>54/71/72</u>	1
<u>11</u>	1	35/52	1	33/45/51	1		
<u>42</u>	1	39/ <u>55</u>	1	39/ <u>62/81</u>	1		
<u>53</u>	2	39/ <u>70</u>	1	51/ <u>54/62</u>	1		
<u>55</u>	1	39/ <u>72</u>	2	51/52/ <u>84</u>	1		
<u>61</u>	1	39/ <u>81</u>	1	51/ <u>54/66</u>	1		
<u>62</u>	1	51/ <u>54</u>	1	51/59/ <u>73</u>	1		
<u>67</u>	2	51/ <u>62</u>	1	52/ <u>55/61</u>	1		
<u>70</u>	2	51/ <u>61</u>	1	52/ <u>6/72</u>	1		
<u>72</u>	2	52/ <u>84</u>	2	52/ <u>62/CP6108</u>	1		
<u>84</u>	1	52/59	1				
		52/ <u>72</u>	1				
		52/ <u>53</u>	1				
		56/ <u>62</u>	1				
		56/ <u>54</u>	1				
		56/ <u>81</u>	1				
		58/59	1				
		59/ <u>53</u>	1				
		59/ <u>73</u>	1				
		68/ <u>42</u>	1				
		68/ <u>84</u>	1				

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