

# Prevalence of Cervical Infection and Genotype Distribution of Human Papilloma Virus Among Females in Da Nang, Vietnam

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**Abstract.** *Aim: The goal of the present study was to determine the prevalence and distribution of high-risk human papilloma virus (HPV) genotypes in women from two districts in and around Da Nang city, Vietnam. Materials and Methods: All participants were randomly selected, 200 from the Hai Chau district and 200 from the Son Tra district. The detection and genotyping of HPV were performed by real-time polymerase chain reaction (PCR) technique. Results: Out of a total of 400 women, we found that 38 (9.5%) were infected with a high-risk HPV genotype, the most prevalent genotypes being 16, 18, 58 and 59. By assessment of the HPV findings in relation to sociodemographic characteristics, we found significant differences between the two study districts and between the age groups, as well as differences associated with occupation and the use of contraceptives. Conclusion: The proportion of high-risk genotypes other than 16 and 18 was relatively high, and since the HPV genotype distribution is known to vary greatly across populations, the information from this study can be used for planning of screening and vaccination programs in Da Nang.*

The human papillomavirus (HPV) is one of the most common causes of sexually transmitted disease in both

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women and men all over the world. Studies on the etiology of cervical cancer have shown that approximately 99% of cases are associated with HPV infection (1, 2).

More than 200 genotypes of HPV have been identified and are generally classified as high-risk and low-risk HPV based on their association with premalignant and malignant lesions (3). The high-risk types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59 have been detected in 99% of all cervical cancer, with approximately 70% of all invasive cervical cancer cases being due to HPV types 16 or 18 (2, 4).

Prophylactic vaccination against HPV has been conducted in many countries for the prevention of cervical cancer. There are a number of variants such as monovalent (HPV16), bivalent (HPV16 and 18), quadrivalent (HPV6, 11, 16 and 18) and nonavalent (HPV6, 11, 16, 18, 31, 33, 45, 52 and 58) vaccines (5, 6). In Vietnam, the measures for cervical cancer prevention include, primary screening for HPV and vaccination against HPV (7). It has been shown that there are differences in the genotype distribution in women with multiple HPV infections not only among women of different ethnicity but also women from different regions (8). Moreover, the tendency of HPV genotypes to cluster in multiply infected women has been frequently observed (8). Knowledge about the type-specific distribution and the prevalence of HPV cervical infection in women from diverse areas and ethnicity is a prerequisite for the planning of an efficient and adequate screening and vaccination strategy with the best possible outcome. Some studies have been conducted within Vietnam, especially in Ho Chi Minh City and Hanoi (7, 9-11), but also in populations from Thai Nguyen, Hue and Can Tho (9). These studies showed a prevalence of high-risk HPV infection, ranging between 2-11%.

There are limited epidemiological data on the prevalence of HPV in cervical infection and distribution of HPV types

in cohorts resident in and around the city of Da Nang, Vietnam. Therefore, the aim of the present study was to examine HPV prevalence and HPV genotype distribution, based on real-time polymerase chain reaction (PCR), in cervical swab samples from women in Da Nang represented by the Hai Chau and the Son Tra districts.

## Materials and Methods

*Study population and sample collection.* In this study, we used cervical samples from 400 women participating in a HPV-screening program in Da Nang city in 2015. Married, non-pregnant women aged 18-49 years were included. Exclusion criteria were a) treatment for gynecological diseases/symptoms during the previous 7 days, b) on-going menstruation, c) sexual intercourse during the 3 days prior to sampling, e) psychiatric disorder. Informed consent was obtained from each participant and personal interviews were performed which included information on sociodemographic characteristics including age, occupation, education and use of contraceptives.

All participants were randomly selected, 200 from the Hai Chau district and 200 from the Son Tra district. Da Nang has a total of seven districts with 56 wards. The Hai Chau district has 13 wards and the Son Tra district has seven wards. In each district, participants between 18-49 years of age were sampled, and the probability of being selected was proportional to the size of the associated ward.

The samples were randomly selected from the list of eligible women by a computer-based program.

*Ethics approval and consent to participate.* Informed consent to use the results of the analysis was obtained from each of the study participants. The study was approved by the Ethics Committee of Da Nang University of Medical Technology and Pharmacy (01/QD-DHKTYDDN-HDDYS).

*HPV detection and typing.* Cervical swab samples were dissolved in 1 ml of 0.9% NaCl and stored at  $-20^{\circ}\text{C}$  until analysis. Genomic DNA from 200  $\mu\text{l}$  of sample solution was extracted by DNA Mini Kit Invisorb<sup>®</sup> Spin Virus 0814 (Sacace, Biotechnologies, Como, Italy) according to the manufacturer's protocol. The concentration and quality of genomic DNA was checked by a NanoDrop 2000 spectrophotometer (Thermo Scientific, Columbia, USA) and 1% agarose gel electrophoresis.

HPV DNA genotypes were identified by real-time PCR using 14 HPV Genotypes Real-TM Quant Kit (Sacace Biotechnologies) for 14 high-risk types (namely types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) and analyzed on Stratagene Mx3000P system (Stratagene, San Diego, CA, USA). All processed samples were analyzed at the Laboratory of Molecular Biology, Dan Nang University of Medical Technology and Pharmacy.

*Statistical analyses.* In order to achieve high statistical power, the sample size was calculated according to Charan *et al.* (12). With the estimated prevalence of HPV infection in women in the community being about 12%, the required minimum sample size was calculated to be approximately 163 under the conditions of 5% Type 1 error and the reliability of the study 95%.

Differences in the prevalence of HPV DNA between the groups were analyzed using the chi-squared test. Statistical analysis was

performed using SPSS Statistics software (version 19, 2012; SPSS, Chicago, IL, USA). Results were considered significant at  $p < 0.05$ .

## Results

Out of a total of 400 women, we found that 38 were infected with a high-risk HPV genotype, thereby exhibiting an overall prevalence of 9.5%. Sociodemographic characteristics and the prevalence of high-risk HPV infection of the study subjects are presented in Table I. Thirteen different HPV types were detected among the 38 HPV-positive women, the most prevalent types being 16, 18, 58 and 59 (Table II).

Single HPV type infection was detected in 24 HPV-positive cases (63.1%) and multiple infection with two, three and four HPV types was detected in 26.3%, 5.3% and 5.3%, respectively.

By assessment of the HPV findings in relation to demographic characteristics, we found significant differences between the two study districts and between age groups, as well as differences associated with occupation and the use of contraceptives. However, we found no association between HPV prevalence and educational level (Table I). When we analyzed the age-specific prevalence we found significantly higher HPV prevalence in women aged 18-29 years compared to women 30-39 years ( $p < 0.001$ ) and women 40-49 years ( $p < 0.006$ ), respectively.

## Discussion

In this study, we investigated a broad spectrum of 14 high-risk HPV genotypes, including the most common types of HPV16 and 18, and found 13 high-risk types among the cervical samples collected from women in Da Nang city. We report a prevalence of 9.5% of cervical HPV infection in Da Nang city, which is at the upper end of range of 2% to 11% previously reported from other cities in Vietnam (7, 9-11). Considering the Hai Chau district, a downtown area with offices, schools, amusement parks and business centers, the prevalence (15.0%) of HPV infection was found to be significantly higher in comparison with women in the Son Tra District (4.0%), which is a new developing area located of the coast and dominated by fishermen, ordinary workers and tourism.

As expected, we found that the most common genotypes HPV16 and HPV18 dominated among the 38 HPV-positive women in Da Nang, a finding that is similar to previous results reported from other places in Vietnam (10) and in other countries (4, 13, 14). About half of the 38 HPV-positive women in Da Nang were infected with only HPV 16 or 18, or both, and the rest with other risk types of HPV. This finding corroborates a previous study of women in the nearby city of Hue (7). Moreover, our data indicate a high prevalence of HPV59 in comparison to earlier reports from

Table I. Demographic characteristics and prevalence of cervical high-risk human papilloma virus (HPV) infection in the study group of 400 women in Da Nang, Vietnam.

Factor	Subgroup	No. of cases	Prevalence, n (%)		p-Value
			HPV-positive	HPV-negative	
District	Hai Chau	200	30 (15.0)	170 (85.0)	<0.001
	Son Tra	200	8 (4.0)	192 (96.0)	
Age (years)	18-29	92	20 (21.7)	72 (78.3)	<0.001
	30-39	188	14 (7.4)	174 (92.6)	
	40-49	120	4 (3.3)	116 (96.7)	
Occupation	Employed	278	20 (7.2)	258 (92.8)	0.017
	Unemployed	122	18 (14.8)	104 (85.2)	
Education	High school or less	238	22 (9.2)	216 (90.8)	0.832
	College degree or higher	162	16 (9.9)	146 (90.1)	
Condom use	Yes	294	22 (7.5)	272 (92.5)	0.022
	No	106	16 (15.1)	90 (84.9)	

Vietnam (9). Thus, we found a high prevalence of HPV genotypes other than 16 and 18, and other studies have shown that the genotype distribution varies considerably across populations worldwide (15). This reflects the importance of knowing the regional genotype-specific distribution and the prevalence of HPV cervical infection in women when designing screening programs and planning vaccination strategies. Moreover, these findings are important for the assessment of the impact of intervention models with the purpose to control cervical cancer incidence.

In a substantial percentage of HPV infections, two or more genotypes may be found and 20-50% of the infected women carry more than one genotype (16). Our study revealed a prevalence of 36.9% of infection with multiple high-risk HPV types in cervical samples from the 38 HPV-positive women in Da Nang. Pham *et al.* demonstrated 41.6% and 20.0% prevalence of multiple-type HPV infection in HPV-positive women in Ho Chi Minh City and Hanoi, respectively (10). It has been shown that there are differences in the genotype distribution in women infected with multiple HPV types between women from different regions (8). Whether our findings reflect such differences must be investigated in a larger number of HPV-positive women.

Our data about the age-specific prevalence of HPV infection among women in Da Nang show a significant decline related to higher age. This is consistent with another study from Ho Chi Minh City (10). The results may be explained by a dynamic population, especially among the young in Da Nang, in combination with sexual transmission during the start of sexual activity. HPV acquisition is generally more common in younger women with multiple sexual partners and many HPV infections are transient (17-19).

Table II. High-risk human papilloma virus (HPV) genotypes detected in cervical samples from 38 HPV-positive women in the study group of women in Da Nang, Vietnam.

Genotype	No. of cases	Prevalence (%)
16	12	31.6
18	8	21.1
31	2	5.3
33	2	5.3
35	4	10.5
45	2	5.3
51	4	10.5
52	4	10.5
56	4	10.5
58	6	15.8
59	6	15.8
66	2	5.3
68	2	5.3

Studies have suggested that the use of condoms has little, if any, protective effect against HPV infection (20, 21). However, our study showed that condom use was associated with a significantly lower prevalence of high-risk type HPV, which was also previously reported from other populations (11, 22, 23).

We found a significantly higher prevalence of HPV infection in unemployed women compared to employed women. However, the HPV prevalence was not associated with the level of education. Interpretation of these data is delicate, but there may be connections with lifestyle and sexual behavior. Other studies have also found somewhat contradictory results and indications of gaps existing between women's knowledge and practice. Ramamurthi *et*

al. found for example that the awareness of cervical cancer, available screening tests and vaccines was higher among employed women than among unemployed, but that unemployed women were more likely to adhere to screening and vaccination programs once provided with the appropriate information (24).

## Conclusion

We found an overall HPV prevalence of 9.5% in Da Nang city, but also significant differences between the two study districts that could possibly be associated with socioeconomic factors. The proportion of high-risk genotypes other than 16 and 18 was relatively high, and since the HPV genotype distribution is known to vary greatly across populations worldwide, it is important to take regional data into consideration in the planning of screening and vaccination programs. In the future, additional studies are warranted, using larger number of samples, in order to confirm the HPV prevalence and distribution in this study and also to relate the data to the incidence of cervical cancer.

## Competing Interests

The Authors declare that they have no competing interests.

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