Genotypic distribution and prevalence of human papillomavirus infection in an apparently healthy female population in Bangladesh

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ABSTRACT

Objective: Human papillomavirus (HPV) comprises around 120 genotypically related viruses, classified into low- and high-risk HPVs, which are capable of replicating inside the keratinocytes of skin or mucous membranes. Studies suggest that infections with HPV-16 or HPV-18 have a higher rate of developing cancer. The aim of our study was to detect HPV early, and to estimate the genotype-specific prevalence of HPV in apparently healthy and asymptomatic females in Bangladesh.
Method: After cervical swab specimen collection, a VIA test was performed to identify any type of abnormality in the cervix. A multiplex PCR amplification of HPV DNA, using L1 consensus primer systems, was performed with type-specific primers, followed by sequencing to detect HPV genotypes.
Result: Of the 417 females, 121 were found to be HPV positive. The most prevalent high-risk HPV genotypes were found to be HPV-16 and HPV-18. Different patient demographic parameters, such as age, socioeconomic status, education, and history of first intercourse, were also studied to establish correlations with HPV infection.
Conclusion: Our results might provide some insights into factors that influence the development of cervical cancer. They might also help in guiding better patient management, increased public health awareness, further testing, and the implementation of existing vaccines.

Introduction

Human papillomavirus (HPV), the biological agent associated with cervical cancer (CC), was first discovered during the 1950s by a group of scientists from different countries (Jawetz et al., 2001). It comprises a large group of double-stranded, circular DNA viruses, each having a genome of around 8000 base pairs (Castro and Bussoloti Filho, 2006). So far, around 120 types of papillomavirus have been isolated from humans, and divided into two groups: oncogenic and non-oncogenic (Rolón et al., 2000; Bernard et al., 2010). Oncogenic HPV genotypes are divided into low-risk or high-risk groups, depending on their oncogenic potential. The low-risk subtypes, such as HPV-6, -11, and -43, are associated with hyperplastic lesions; these have little to no oncogenic risk. In contrast, high-risk genotypes, such as HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, and 68, are considered to be associated with 96.6% of invasive CCs (Muñoz et al., 2003).

Bangladesh has a population of 58.7 million women aged 15 or above, who are at risk of developing CC (Bruni et al., 2019). Current estimates indicate that, every year, 8068 women are diagnosed with CC and 5214 women die from this disease (Bruni et al., 2019). After breast cancer, CC is the second most common cancer in women of Bangladesh (Islam, 2014). More than 80% of patients diagnosed with this eminently preventable cancer are in clinically advanced, inoperable stages (Basu et al., 2010). According to the Global Alliance for Vaccines and Immunization (GAVI), Bangladesh stands at 11th in the world in terms of CC fatalities, with 17.9 per 100 000 women dying every year due to this mainly sexually transmitted disease (Li et al., 2006). In Southern Asia, which includes Bangladesh, around 7.9% of women in the general population are estimated to harbor cervical HPV infection at a given time, with 82.8% of invasive CCs attributed to HPVs 16 or 18.

Furthermore, while establishing the relationship between CC and exposure to human papillomavirus (HPV), several patient-specific features, such as selected aspects of sexual and reproductive behavior, use of oral contraceptives, screening practices, smoking, age at first inter-

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course, and husband's professional status, were also considered as potential risk factors (Bosch et al., 1992; Bosch et al., 1996).

However, an exact population-specific estimation of the HPV burden in Bangladesh remains elusive. Since infection with distinct types of human papillomavirus (HPV) is the major etiological factor in developing CC, our study sought to establish the epidemiology of HPV in Bangladesh. To address this, a population-based study on healthy Bangladeshi women was first performed, in order to identify the prevalence of high-risk HPV genotypes. Furthermore, the significance of different patient-specific risk factors, which might be facilitating HPV infections and eventually leading to CC, was also examined.

Materials and methods

Patient selection

Our investigation involved samples from the hospitals or health centers of 14 multi-centered regions of Bangladesh. All apparently healthy women attended a gynecological outpatient clinic for CC screening. In total, 410 women aged 14–70 years (median age 30 years) were assessed between February 2015 and June 2018. These women underwent gynecological examination, ThinPrep cytology testing, and HPV DNA testing (Zhao et al., 2011). The inclusion criteria for these women were as follows: (1) sexually active; (2) of all economic classes; and (3) not pregnant at the time of enrollment. The exclusion criteria were: pregnancy; acute genital inflammation; cervical or total uterus resection; and immune deficiency disease.

Cervical swab sample collection

Cervical cells were collected by using a cytobrush and kept in PreservCyt® solution (a commercially available methanol-based preservative) from Bangabandhu Sheikh Mujib Medical University (BSMMU). The collected samples were kept at −20°C until HPV DNA detection and genotyping were performed.

DNA extraction

Extraction of total DNA from cervical samples was performed using a QIAamp® DNA Mini Kit (QIAGEN, Germany), according to the manufacturer's instructions. For long-term storage of DNA, eluting in Buffer AE and storing at −20°C was recommended.

Detection of HPV and genotyping

Genotyping of HPV was carried out to identify whether the virus present in the sample was in the high-risk or low-risk group. For detection of HPV, the conserved L1 region (encoding for a major capsid protein) was targeted for PCR (polymerase chain reaction), following the workflow described in our previous study (Sharmin et al., 2021). The MY11/MY09 primer pair-mediated PCR (MY-PCR) and the GP5/GP6 primer pair-mediated PCR (GP-PCR) are the most frequently used amplification systems for the detection of HPV DNA in clinical samples. Recent studies indicate that any single method for HPV detection may underestimate the true prevalence of HPV in cervical samples (Qu et al., 1997).

Statistical analysis

Statistical analyses were performed using Graphpad Prism v8 for Windows (GraphPad Software, San Diego, California, USA) and IBM SPSS v27 for Windows (SPSS Inc., Chicago, USA). The prevalence of HPV infection, genotype distribution, and the presence of single or multiple HPV infections were analyzed separately. For the analysis of associations between patient demographic features and HPV infection, either Fisher's exact test (Fisher, 1992) or the chi-square test (McHugh, 2013)

was used. Only those correlations with p-values < 0.05 were considered statistically significant.

Results

HPV-16 and HPV-18 were the most commonly detected HPV subtypes among infected asymptomatic women

In total, 410 apparently healthy females (average age 36.95 ± 0.764 years) were included in the study. Positive HPV test results were obtained for 121 individuals, and negative results for the other 289, representing a prevalence of HPV infection in Bangladesh of 41.86% (Tables 1 and 2). Notably, of the HPV-positive women, 75 were positive for a single HPV subtype, accounting for 61.98% of HPV infections and 18.29% of all samples; 46 individuals were positive for multiple HPV subtypes, accounting for 38.01% of HPV infections and 11.21% of all samples (Table 1). Both single infections and multiple infections can be major aspects of disease progression to CC.

High-risk HPV genotypes are more prevalent among middle-aged women

Overall distributions of HPV-infected individuals in the different age ranges were evaluated, considering both single and multiple infections. The levels of involvement of low-risk and high-risk HPV genotypes for each age range were also assessed. Statistically significant differences ($p < 0.001$) were found between the different age groups in terms of overall HPV infections (Table 2). The prevalence of HPV infections was significantly higher for women aged 20–39 years, with no infections found in women aged over 60 years (Table 2). We observed that the prevalences of high-risk HPVs, either with one or multiple genotypes, were higher amongst the middle-aged women (aged between 30 to 49) compared to the other age groups (Table 2).

Women with a history of teenage sexual intercourse had a higher frequency of HPV infection

When other factors were analyzed, such as HPV infection status and age of first intercourse, it was found that women who became sexually active at a young age (12 to 20) had a higher chance of becoming infected with HPV (Table 3). Notably, it was also observed that women who started their sexual life in their late 20s has less chance of infection with HPV.

Uses of contraceptives might have an influence on HPV infection susceptibility

With regard to the use of contraceptives, 52.83% of women who had always used OCPs were found to have been infected with HPV, whereas only 1.88% of women who used condoms became infected (Table 4). This observation is to be expected, since practicing safe sexual intercourse is most likely to prevent infections among women. Moreover, 43.91% of HPV-negative women had always used OCPs, whereas only 5.2% of HPV-negative women had never used contraception (Table 4).

Socioeconomic conditions might influence the acquisition of HPV infection

When evaluating other major cofactors of HPV infection among women, such as socioeconomic status and educational status, no significant differences were observed. However, women who were comparatively less educated and those who belonged to the lower-middle economic class did show a higher likelihood of HPV infection (Tables 5 and 6). Moreover, the occupations of the husbands of HPV-infected women might be an important factor in transmitting the virus from other infected women to their wives (Table 7). For instance, the wives of self-employed husbands were more likely to be HPV-positive compared with other groups (Table 7).

Table 1

Detection of HPV in asymptomatic individuals in Bangladesh

HPV Genotypes	HPV-16	HPV-18	HPV-16 and HPV-18	HPV 16 and HPV-69	HPV-33 and HPV-6	HPV-70	HPV-31	HPV-90	HPV-62
Number of asymptomatic individuals	3	31	20	2	1	1	1	1	1
Out of total tested HPV-positive asymptomatic individuals (%)	2.479	25.619	16.528	1.652	0.826	0.826	0.826	0.826	0.826
Out of total tested asymptomatic individuals (%)	0.731	7.56	4.878	0.487	0.243	0.243	0.243	0.243	0.243

Table 2

The overall distribution of HPV-infected individuals in the different age ranges

Infection/age group	< 20	20–29	30–39	40–49	50–59	> 60	p-value (Fisher's exact test)
HPV −ve	3	33	205	38	8	3	<
HPV +ve	1	17	49	48	5	0	**0.001**
Single-type HPV infection							
With only one low-risk HPV	0	0	3	3	0	0	0.6049
With only one high-risk HPV	0	17	25	24	3	0	
Total	**0**	**17**	**28**	**27**	**3**	**0**	
Multiple-type HPV infection							
With two low-risk HPVs	0	0	0	1	0	0	0.5724
With one high-risk and one low-risk HPV	0	0	3	5	0	0	
With two high-risk HPVs	0	0	20	15	2	0	
Total	**0**	**0**	**23**	**21**	**2**	**0**	

Table 3

The status of HPV infection of women, considering their age at first intercourse

	Number of HPV +ve individuals	Number of HPV −ve individuals	p-value (Fisher's exact test)	Relative risk (95% CI)
Aged between 10 and 19	83	138	0.7105	1.034
Aged 20 and above	38	151		(0.8860–1.270)

Table 4

Contraceptives used by the women during intercourse, and their HPV-infection status

Contraceptive used	No of HPV +ve patients	No of HPV −ve patients	p-value (Fisher's exact test)
Condom	1	9	0.1603
Injectables	5	18	
OCP	28	83	
Withdrawal method	6	10	
Intra-uterine device	1	3	
Tubectomy	2	1	
Vasectomy	0	1	
OCP and condom	0	1	
No contraceptives	10	63	

Table 5

Association of socioeconomic status of women with their HPV infection status

Socioeconomic condition	No of HPV +ve cases	No of HPV −ve cases	p-value (χ^2 test, df)
Higher middle	18	85	0.3085
Lower middle	21	57	(2.352,
Poor	14	48	2)

In cases of women with clinical symptoms, such as lower abdominal pain, anorexia, presence of cysts, malaise, bleeding between periods, or pain during intercourse, none of these symptoms was found to show a strong correlation with HPV infection (Table 8).

Discussion

Around 99.7% of invasive cervical cancer (CC) cases worldwide are associated with infection by HPV (Walboomers et al., 1999). HPV DNA can be detected in nearly all cervical carcinomas, with higher HPV prevalence rates found with increasing severity of cervical dysplasia (Husman et al., 1994). Due to the strong correlation between HPV and CC, various efforts have been made to improve the sensitivity of methods used to detect HPV types in samples from women with abnormal genital cytology. HPV detection and typing has become an essential tool for the diagnosis and management of HPV-related disease (Husman et al., 1994; Cope et al., 1997; Hildesheim et al., 1998). Detection of HPV genotypes, whether high risk or low risk, helps to determine the chance of developing CC (Meijer et al., 2006).

CC ranks as the second most frequent cancer among women between 15 and 44 years of age in Bangladesh (Bruni et al., 2019). In Southern Asia, around 7.9% of women in the general population are estimated to harbor a cervical HPV infection at a given time, with 82.8% of invasive CCs attributed to HPV-16 or 18 (Bruni et al., 2019). Our results showed the HPV prevalence in asymptomatic women to be 29.51% in Bangladesh, with a prevalence of high-risk HPV of 19.53% (including high-risk and low-high-risk women).

Table 6
Association of educational status of women with their HPV infection status

Educational status	No of HPV +ve patients	No of HPV −ve patients	p-value (Fisher's exact test)
Illiterate	10	41	0.6551
Primary	23	67	
Secondary	15	56	
Higher secondary	4	24	
University graduate	1	2	

Table 7
Association of husbands' occupations with the HPV infection status of women

Husbands' occupation	HPV −ve	HPV +ve	p-value (χ^2 test)
Self-employed	39	20	0.001425
Service holder	52	10	
Laborer	24	5	
Farmer	44	8	
Teacher	4	0	
Retired	1	1	
Tailor	1	1	
Foreign	2	1	
Rickshaw puller	3	0	
Driver	5	1	
Jobless	6	1	
Disabled	2	0	
Divorced	1	1	
Conductor	1	1	
Died	1	2	

The distribution of HPV subtypes also showed regional differences. A previous study indicated that HPV-16, 18, 31, 33, and 58 are the most prevalent in Europe, whereas in North America, HPV-16, 53, 52, 18, and 39 are the most common subtypes (Crow, 2012). The most common subtypes in Africa were found to be HPV-16, 52, 18, 58, and 31, while in Asia the most prevalent were HPV-16, 52, 58, 18, and 56 (Crow, 2012). The HPV-16 and 18 subtypes are the most commonly found worldwide, whereas the HPV-33, 45, 52, and 58 subtypes are more prevalent in Asia than elsewhere in the world, especially in Japan and China (Asato et al. 2004; Chen et al., 2009). In our study, the prevalent high-risk subtypes were HPV-16, 18, and the combination of HPV-16 and 18; the low-risk genotypes HPV-6, 70, 31, 33 and 69 were also detected, but in very low numbers. The overall HPV prevalence for our study population was 29.5%. Infection with multiple subtypes accounted for 18.18% of HPV infections and 5.36% of all samples. Based on our findings, HPV screening systems in Bangladesh should focus on HPV-33, 31, 69, and 70, in addition to HPV-16 and 18.

When demographic data were analyzed, correlations between HPV prevalence and factors such as age of women, their educational level,

and occupations of husbands were observed to be significantly associated. However, no significant associations were observed between HPV prevalence and the age of women during first intercourse, socioeconomic status, and use of contraceptives.

The distribution of HPV subtypes among infected individuals in different age ranges has been previously reported, with high-risk HPV infections (including low-high-risk and high-high-risk women) accounting for 37.19% of HPV infections and 10.9% of all samples (Crow, 2012). Our study showed that the distribution of HPV subtypes and their prevalence exhibited age-related differences; a similar observation was previously reported in a study conducted on Chinese women (Zhong et al., 2017). Several conclusions can be inferred from the distributions of each high-risk HPV type in relation to age (Sargent et al., 2008). Two of the most common age ranges for HPV infection were those under 14 and those over 60 years old; these findings were similar to those observed in previous studies in China (Chen et al., 2009). The main reasons may be the immature immune system against HPV found in young females, and the physiological and immunological disorders associated with hormone fluctuations during the menopausal transition of older women. Menopausal stage has also been found to be associated with HPV detection, with increased prevalence among perimenopausal compared with premenopausal women (Althoff et al., 2009).

A previous study has suggested an association between early-age sexual intercourse and cervical neoplasia in women (Bosch et al., 1996). Our study showed a similar trend among the women in our cohort, with those having a history of early-age intercourse (< 20 years) being more vulnerable to HPV infection compared with those becoming sexually active later in life.

Bosch et al. also reported that the presence of HPV DNA in husbands conveyed a fivefold risk of CC among their wives, and reported that men having multiple sexual partners due to their occupations and lifestyles were carriers of HPV DNA, possibly of high-risk HPV subtypes, and thus may place their wives at risk of developing CC (Bosch et al., 1996). Our study did not explore the association between the number of sexual partners of the husbands and the HPV status of their wives. Instead, the professions of the husbands were considered in order to establish whether these could influence the HPV infection risk among their wives. Interestingly, the wives of self-employed husbands were observed to be

Table 8
Clinical symptoms of women and their associated HPV infection status

Clinical symptoms		HPV infection status		Odds ratio (OR)	95% CI	p-value (Fisher's exact test)
		HPV +ve	HPV −ve			
Pain in lower abdomen	Yes	22	99	1.009	0.5221–1.924	> 0.9999
	No	24	109			
Anorexia	Yes	3	10	1.381	0.3936–5.056	0.7098
	No	43	198			
Presence of cyst	Yes	4	12	1.556	0.5266–4.567	0.5008
	No	42	196			
Malaise	Yes	2	15	0.5848	0.1289–2.293	0.7452
	No	44	193			
Blood between periods	Yes	5	25	0.8927	0.3542–2.359	> 0.9999
	No	41	183			
Pain during intercourse	Yes	0	4	0	0.000–4.642	> 0.9999
	No	46	204			

more prone to HPV infection, suggesting the probability that their husbands might be having multiple sexual partners, and thus spreading the infection to their wives. In order to achieve a clearer correlation, more specific and targeted information on husbands' sexual behaviors needs to be incorporated.

Our results suggested some association between HPV infections and the ages of asymptomatic women. However, the total samples for those aged < 20 and for those > 60 were much lower than those for the other age groups, which might have led to the prevalence data for both these age groups being unreliable. Additionally, a larger overall sample size would be needed to reach conclusive evidence for the entire population of Bangladesh. The relatively small sample size may also explain the non-significant associations between most of the patient demographic factors and HPV infection. Although we are not aware of the number of remaining undiagnosed women in our region with HPV, the results from our study might help to clarify the epidemiological associations of HPV infection in the asymptomatic women of Bangladesh.

Conclusion

Our study showed that HPV prevalence varies significantly among the apparently healthy Bangladeshi females from different age groups. These results can serve as a valuable reference to guide CC screening and HPV vaccination programs in Bangladesh. Additionally, HPV testing in CC screening programs has clinical significance for women under 20 and over 60 years of age, while the development of a vaccine targeting HPV-16, 18, 33, 52, and 58 subtypes remains important. Our research supports previous studies in recommending continued educational campaigns focusing on the safety of the HPV vaccine, and its efficacy in reducing CC.

Ethical approval and consent

Institutional review board (IRB) clearance was granted by Bangabandhu Sheikh Mujib Medical University (BSMMU), Shahbag, Dhaka (No. BSMMU/2017/151). The Ethical Clearance Committee of the University of Dhaka also approved all protocols (Ethical Clearance No. 30/Bio.Fac./2016–2017). Written consent was obtained from all patients before the collection of tissue samples.

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Author contributions

MY conceptualized and designed the study. AN provided the samples. SS, BS, and AA collected the samples and performed genotyping and demographic studies. SS and MAAKK performed the statistical analyses. SS, MAAKK, CRA, and MY wrote the manuscript. All authors read and approved the final manuscript. BS, AA, and MAAKK contributed equally as co-second authors.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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