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RESEARCH PAPER

Distribution of High-risk Human Papillomavirus Genotypes Among Woman with Cervical Precancerous Lesions Attending in Tertiary Care Hospital of Bangladesh

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Abstract

Background: Human papillomavirus (HPV) is the most frequent cause of high-grade lesions and carcinogenesis such as cervical intraepithelial neoplasia (CIN) and cervical cancer (CC). However, the prevalence and genotype distribution of HPV infection varies greatly with respect to geographical regions. Determining the prevalence and distribution of HPV in a particular area is, therefore, of immense significance.

Objectives: This study was undertaken to evaluate the distribution of high-risk HPV genotypes in cervical precancerous lesions.

Methods: This descriptive cross-sectional study was conducted at the Colposcopy Clinic in the Department of Gynecologic Oncology, Bangladesh Medical University, Dhaka over a period of 6 (six) months from November 2022 to April 2023. A total of 151 women aged between 30-70 years with Colposcopically abnormal and histopathologically proven precancerous lesions of the cervix were purposively included in the study. HPV-DNA test was done by US FDA-approved Digene's Hybrid Capture II (HCII) technology. However, the high-risk (HR) HPV DNA was assessed by PCR testing.

Result: In the present study majority (81.5%) of patients had low-grade CINs (CIN1), and the rest (18.5%) had high-grade lesions (CIN2 & CIN3). Over half of the patients tested positive for HPV DNA. The predominant genotype harbored by the patients was HPV16 (63.2%). The genotype HPV18 alone was negligible (3.3%6). The HPV16 and HPV18 together comprised 6.6%. While HPV 16 in association with other genotypes comprised 6%, HPV18 associated with other genotypes was the least (0.7%). About two-fifths (39%) of the low-grade CINs (CIN1) and all of the high-grade CINs (CIN2 & CIN3) were tested positive for HPV ($p < 0.001$). No significant association was found between HPV-DNA genotypes and CIN grade

Conclusion: The majority of women with CIN had low-grade lesions. Over half of the CIN patients have positive HPV-DNA test results. While HPV16 is the most prevalent genotype among the patients, the HPV18 genotype is uncommon. The prevalence of other genotypes are rare. All of the high-grade CINs and two-fifths of the low-grade CINs are found to be HPV-positive. Nevertheless, there is no significant association between CIN grade and HPV-DNA genotypes.

Keywords: High risk Human papilloma virus genotype, precancerous lesion of cervix, high grade CIN lesion, low grade CIN lesion.

Introduction

According to Globocan (2020), cervical cancer is the second leading cause of morbidity and mortality with an estimated 19.3 million new cancer cases and almost 10.0 million cancer deaths occurred in 2020.[1] Human papillomavirus (HPV) is one of the most common causes of sexually transmitted diseases in both men and women worldwide and is thought to be the most common sexually transmitted viral disease in the United States. Genital HPV infection is not a reportable disease, so actual incidence and prevalence figures are not known; however, it is estimated that the incidence of new infections in the United States ranges from 1 million to 5.5 million per

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year, and the prevalence is estimated to be as high as 20 million.[2] HPV continues to be an important topic, as rates of infection appear to continue to be rapidly increasing. Cervical cancer is the third most common cancer in women in the United States, preceded by skin cancer and breast cancer as the first and second most common respectively.[3] In developing countries, cervical cancer is often the most common cancer in women and may constitute up to 25% of all female cancers.[4]

The link between genital HPV infections and cervical cancer was first demonstrated in the early 1980s by Harold zur Hausen, a German virologist. Since then, the link between HPV and cervical squamous cell carcinoma has become well-established. The magnitude of the association between HPV and cervical carcinoma is higher than that for the association between smoking and lung cancer.[5] In 1996, the World Health Organization, along with the European Research Organization on Genital Infection and Neoplasia and the National Institutes of Health Consensus Conference on Cervical Cancer, recognized HPV as an important cause of cervical cancer. Scientists have identified about 30 HPV types that are spread through sexual contact and infect primarily the cervix, vagina, vulva, penis, and anus. Of these, four are most often found within the malignant cells of cervical cancers, with type 16 accounting for about half of the cases in the United States and Europe and types 18, 31, and 45 accounting for an additional 25 to 30% of cases.[4] HPV has been implicated in 99.7% of cervical cancer cases worldwide.[6] Adenocarcinomas of the cervix are also related to HPV, but the association is less pronounced and is age-dependent (Andersson et al., 2001). In women younger than 40 years, HPV was present in 89% of adenocarcinomas, whereas in women aged 60 years and older, HPV was observed in only 43%.

Human papillomaviruses (HPV) are a group of remarkably diverse double-stranded and non-enveloped DNA viruses (7 ~ 8 kb long), from the Papilloma viridae family, which are causally involved in the etiology of various benign and malignant neoplastic lesions of mucosal and skin epithelium.[7] Currently, more than 200 different HPV genotypes have been identified. Genotypes HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59 are regarded as high-risk types (hr-HPV) because they are identified in high-grade squamous intraepithelial lesions (HSIL) and invasive cervical cancer tissues.[8,9] On the other hand, the genotypes HPV 6 and 11 are considered as low-risk types.[9,10]

Transmission of HPV occurs primarily by skin-to-skin contact. Epidemiologic studies clearly indicate that the risk of contracting genital HPV infection and cervical cancer is influenced by sexual activity.[11] An individual is at greater risk of becoming infected with HPV if he or she has had multiple sexual partners at any time or is the partner of someone who has had multiple sexual partners.

In addition to sexual activity, age is an important determinant of the risk of HPV infection.[7,12] Most cervical cancers arise at the squamocolumnar junction between the columnar epithelium of the endocervix and the squamous epithelium of the ectocervix. At this site, there are continuous metaplastic changes. The greatest risk of HPV infection coincides with the greatest metaplastic activity. The greatest metaplastic activity occurs at puberty and first pregnancy and declines after menopause. HPV infection is most common in sexually active young women, 18 to 30 years of age. There is a sharp decrease in prevalence after 30 years of age. However, cervical cancer is more common in women older than 35 years, suggesting infection at a younger age and slow progression to cancer. Persistence of infection is more common with the high-risk oncogenic HPV types and is an important determinant in the development of cervical cancer.

Detection of high-risk HPV is necessary but may not be sufficient for the development of cervical cancer. Studies suggest that whether a woman will develop cervical cancer depends on a variety of additional factors that act in concert with cancer-associated HPV types in the process that leads to cervical cancer. The primary immune response to HPV infection is cell-mediated; therefore, conditions that impair cell-mediated immunity such as renal transplantation or human immunodeficiency virus disease increase the risk of acquisition and progression of HPV (16, 17, and 18).

Evidence showed that the prevalence of HPV subtypes is changing over time, possibly in part due to HPV

vaccination in the population. In recent years, there have been different trends in the prevalence of different HPV subtypes. Therefore, it has great significance to analyze the prevalence and distribution of HPV. Genotypes 16, 58, 52, and 18 are among the predominant HPV and HPV infections associated with cervical lesions in China.[13] So regional differences in HPV genotype distribution and the real carcinogenic HPVs need to be discovered in the control and prevention of HPV infections and their sequelae.

Materials and Methods

The study was a descriptive cross-sectional study. Conducted at the Colposcopy Clinic in the Department of Gynecologic Oncology, Bangabandhu Sheikh Mujib Medical University, Dhaka over a period of 6 months from November 2022 to April 2023. A total of 151 women, aged between 30-70 years with colposcopically abnormal and histopathologically proven precancerous lesions of the cervix and non veccinated women, were included as the study population. Colposcopically abnormal but histopathologically normal and unmarried women were excluded from the study.

Signal-amplified techniques for detecting HPV include hybrid capture and branched DNA approaches. In our setting hybrid capture technology (the most widely used technology) was used. To perform the HCII assay, the test used a small brush or spatula to collect a sample of cells from the surface of the cervix, as in a Pap test. The HPV test uses the same method as a Pap test to obtain a cervical sample, but a different test is run on the cells that can determine whether or not a high-risk HPV infection is present. The collected specimens were combined with an extraction buffer to release and denature the target HPV DNA. The released target DNA then combined with specific RNA probes to create RNA-DNA hybrids, which were captured onto a solid phase by an antibody specific to the hybrids.

These captured RNA-DNA hybrids were then tagged with antibody reagents linked to alkaline phosphatase. A chemiluminescent substrate then produced light that was measured on a luminometer in relative light units (RLUs). The amount of light generated is proportional to the amount of target DNA in the original specimen. As with any medical test, the HPV test is not 100% accurate.

Colposcopy was performed in pre-menopausal on days 8-12 of the menstrual cycle and in post-menopausal women it was performed on 10-12 days after estrogen therapy. In patients, where any other procedure was done on the cervix, it was performed after 6 weeks. The patient was advised to take Mefenamic Acid 500 mg tablet or Paracetamol 500 mg tablet 30 minutes before the procedure. The patient was laid down in a lithotomy position. After introducing self-retaining Cusco's speculum in the vagina, the mucus was swabbed off with cotton swabs moistened with normal saline. On speculum examination, the nature of the secretions of the cervix and vagina was noted, including any other obvious findings such as polyp, ectropion, nabothian cysts, inflammation, atrophy and infection, leukoplakia (hyperkeratosis), ulcer, condyloma etc. Green filter was used to evaluate the vascular details to detect any findings suggestive of the precancerous stage like punctuation, mosaic pattern, irregular branching vessels etc. by using higher levels of magnification, noting the borders of the transformation zone. The cervix was swabbed with cotton wool soaked in freshly prepared 5% diluted acetic acid solution for 3 minutes. Epithelium with a high nucleo-cytoplasmic ratio was turned white after acetic acid application. Next, the iodine test was performed by the application of Lugol's iodine solution. Results were interpreted for normal squamous epithelial cells as mahogany brown. The normal columnar epithelium, immature squamous metaplasia, regenerating and inflamed epithelium cells contain very little glycogen and thus, either do not or partially stain with iodine. Findings were noted in Odell's diagram in which colposcopic lesions were represented in a circular diagram in relation to external OS. The results of colposcopy were analyzed as per the scoring system proposed by Reid and Scalzi and IARC.

Colposcopy-guided biopsy materials were taken from the suspicious areas of the cervix. For those with normal colposcopic findings, a biopsy was done randomly within the transformation zone. Hemostasis

was done by applying Monsel's solution to the biopsied area. The findings were documented and explained to the participants. All subjects underwent colposcopy and cervical biopsy on the same day same sitting that the HPV DNA specimens were obtained. Then the association of HR-HPVDNA genotyping and histopathological report were assessed.

Data were collected using a semi-structured questionnaire (research instrument) addressing all the key variables of interest.

Data were processed and analyzed using Statistical Package for Social Sciences (SPSS), version 25.0. The test statistics used to analyze the data are descriptive statistics. The distribution of different types of Human papillomavirus genotypes in cervical precancerous lesions and cancer was assessed using descriptive statistics. HPV-DNA genotyping was judged against the histopathological examination of the colposcopically-directed biopsy material taken from the cervical lesions. Accordingly, the association was helpful in choosing the vaccine type for the Bangladeshi population.

Results

In this study, among 151 women, nearly half (46.4%) of the patients were 40 or below 40 years old, 31.1% 41 – 50 years, 19.2% 51 – 60 years and 3.3% > 60 years old with the mean age of the patients being 43.3 ± 9.2 (range: 30 – 70) years (table I).

Table I: Distribution of study population by their age (n = 151)

Age (years)	Frequency	Percentage
≤40	70	46.4
41 – 50	47	31.1
51 – 60	29	19.2
> 60	5	3.3

*Mean age = 43.3 ± 9.2 years; range: 30 – 70 years.

The majority of (81.5%) of patients had CIN1 (low-grade), 9.9% had CIN2 and 8.6% had CIN3 and this two grade (CIN2 & CIN3) together comprises high-grade lesions (18.5%) (Table II).

Table II: Distribution of patients by the grade of CIN (n = 151)

Grade	Frequency	Percentage
CIN 1	123	81.5
CIN 2	15	9.9
CIN 3	13	8.6

HPV-16 comprised the majority (88.1%) of the genotypes followed by HPV 18 (21%) and other genotypes constitutes 3 (3.9%) cases (table III).

Table III: Distribution of patients by isolated presence of HPV genotypes (n=76*)

Genotypes	Frequency	Percentage	95% CI of p (proportion)
HPV 16	67	88.1	0.809 – 0.953
HPV 18	16	21.0	0.119 – 0.303
Other genotypes	3	3.9	-

* Total will not correspond to 100% for multiple responses.

While over 63% of the patients harbored HPV16 alone, the genotype HPV 18 alone was harbored by a negligible proportion of patients (3.3%). The 95% CI of proportion (p) demonstrates that 81 – 95% of a sample of women with precancerous lesions chosen at random will exhibit genotype HPV 16 and that 12 – 30% will harbor HPV 18 (Table IV).

Table IV: Distribution of patients by genotypes of HPV (n = 76)

Genotypes	Frequency	Percentage
HPV 16 alone	48	63.2
HPV 18 alone	5	3.3
HPV 16 with HPV 18	10	6.6
HPV 16 with others	9	6.0
HPV 18 with others	1	0.7
Other genotypes	3	2.0

All the high-grade CINs (CIN2 & CIN3) were HPV positive as compared to 39% of the low-grade CINs (CIN1) ($p < 0.001$) (Table V).

The Chi-square (χ^2) Test revealed that there was no significant association between CIN grade and HPV-DNA genotypes ($p = 0.252$) (Table VI).

Table V: Association between CIN grade and HPV-DNA status

HPV-DNA	CIN		
	CIN1(n = 48)	CIN2(n = 15)	CIN3(n = 13)
Positive	48(39.0)	15(100.0)	13(100.0)
Negative	75(61.0)	0(0.0)	0(0.0)

*Data were analyzed using the Chi-square (χ^2) Test; figures in the parentheses denote corresponding percentages

Table VI: Association between CIN grade and HPV DNA genotypes

Genotypes	CIN (Cervical Intraepithelial Neoplasia)			p-value*
	CIN1 (n = 48)	CIN2 (n = 15)	CIN3 (n = 13)	
HPV 16	30(62.5)	10(66.7)	8(61.5)	
HPV 18	1(2.1)	2(13.3)	2(15.4)	0.252
HPV 16 with HPV 18	9(18.8)	1(6.7)	0(0.0)	
HPV 16, 18 with others	8(16.6)	2(13.3)	3(23.1)	

*Data were analyzed using the Chi-square (χ^2) Test; figures in the parentheses denote corresponding percentages.

Discussion

Cervical cancer is mostly caused by high-risk human papillomavirus (HR-HPV) infection. Due to significant regional differences in the distribution of HPV genotypes, baseline data on the type-specific prevalence of HPV in a given nation are required in order to assess the efficacy of the HPV-based cervical cancer prevention programs that are currently in place.

The present study demonstrated that the majority (81.5%) of patients had low-grade CINs. For HPV-DNA, more than half of the patients had positive test results. While HPV16 (63.2%) was the most common genotype that the patients carried, the HPV18 genotype was a mere 3.3% of the total. The combined prevalence of HPV 16 and HPV 18 was 6.6%. The presence of other genotypes was negligible. Nearly two-fifths (39%) of the low-grade CINs and all of the high-grade CINs were positive for HPV. Crosstab analysis revealed that there was no significant association between HPV-DNA genotypes and CIN grade. A recent large-scale study conducted in China enrolled 92,932 patients over a period of 5 years from January 2017 to December 2021 and analyzed the prevalence and distribution of HPV genotypes.[21] The results showed that 19.4% of specimens were HPV-positive. The most common genotypes of HPV included five HR-HPV which were HPV52 (4.08%), HPV58 (2.45%), HPV16 (2.38%), HPV53 (1.84%) and HPV51 (1.56%), and one LR-HPV that was HPV81 (1.37%). Li and associates in a study in 2011 demonstrated that HPV16 has the highest incidence of cervical cancer, while the distribution of other types varies regionally in different countries.[14]

Several meta-analyses demonstrated the association of HPV16 and HPV18 with invasive cervical cancer globally, including Asia.[15] In China, the most common HPV types in order of decreasing prevalence were as follows: HPV16, HPV58, HPV52, HPV18, and HPV33.[16] However, a large-scale epidemiological survey of HPV in 51,345 women in China showed that HPV52, HPV16, and HPV18 were the 3 most common types.[17]

The prevalence of high-risk HPV (HR-HPV) infection among Thai women with CIN 2-3 ranged from 64.8% to 90.1%.[18,19] The three most common HR-HPV genotypes noted among Thai women with CIN2-3 were HPV16 (38.5%), HPV58 (20.0%), and HPV18 (5.5%).[19] Zhang and colleagues (2020) in a recent meta-analysis (of 8 studies) demonstrated the prevalence and distribution of HPV genotypes in cervical intraepithelial neoplasia (CIN) in China. A total of 8 studies were identified, which comprised 2950 patients with CIN1 and 5393 with CIN2/3. The overall HPV infection rate was 84.4%. The HPV infection rate was significantly higher in the CIN2/3 group (87.0%) than in the CIN1 group (79.5%) ($p< 0.001$). The study suggested that HPV16, HPV52, and HPV58 were the top three types of CIN in China.[13]

To characterize HPV genotypes in Northeastern Tanzania, a study was carried out on 215 women from the Reproductive Health Clinic at Kilimanjaro Christian Medical Centre. Cervical scrapes and biopsies were obtained for cytology and HPV DNA detection. Over one-third (36.7%) of enrolled participants tested positive for HPV DNA, with a large proportion having multiple infections. The prevalence of HPV infection increased with lesion grade (14% in controls, 67% in CIN1 cases and 88% in CIN2-3). Among ICC cases, nearly 90% had detectable HPV. Overall, 31 HPV genotypes were detected; the three most common HPV genotypes among ICC were HPV16, 35 and 45. In addition to these genotypes, co-infection with HPV18, 31, 33, 52, 58, 68 and 82 was found in 91% of ICC. Among women with CIN2-3, HPV53, 58 and 84/83 were the most common. HPV35, 45, 53/58/59 were the most common among CIN1 cases.[20]

The distribution and prevalence of HPV have been reported to vary by geographic region, and even among different areas in the same country. Geographical differences in HPV distribution may affect the effectiveness of the HPV vaccine in different populations and different age groups. Regional data on the prevalence and type distribution of HPV are essential for estimating the impact of vaccines on cervical cancer and for formulating new vaccination strategies.[21]

Persistent or repeated HPV infection is an important risk factor for cervical cancer and precancerous lesions.[22,23] Infection with HPV subtypes varies at different stages of disease progression. The infection rate of HPV16, HPV18, and HPV52 was significantly higher in the CC group, and the infection rate of HPV58 was higher in the CIN2/3 group. Additionally, the prevalence of HPV73 and HPV83 infection decreased with increasing cervical lesions. Since HPV16, HPV18, and HPV52 are typical HR-HPV subtypes, persistent infection of HR-HPV has been considered an independent risk factor for cervical cancer in women.

CIN is a reversible condition almost 70-90% revert back to normal. So HPV DNA infection is very transient. For developing CIN II and CIN III it needs persistent of HPV infection in cervix and vagina. So in CIN I positive cases maximum precancerous lesions become HPV negative.

While the findings of this study are consistent with some previous reports, they widely differ from the reports of some other studies. Moreover, in this study, there were only 20 (26.3%) cases of CIN had HPV multi-infection. Our results indicate that infection and prevalence of HPV subtypes differ in their prevalence and role in the disease process. Regular analysis of HPV infection and genotype distribution is necessary to understand human infection and its trends, which is essential to deciding the types of vaccines to be effective in a particular geographic region as well as to develop new ones.

Conclusion

From the findings of the study, it appears that the majority of women with CIN had low-grade lesions. Of the CIN patients, over half show positive test results for HPV-DNA. While HPV16 is the most common genotype that the patients harbored, the HPV18 genotype is rarely found. Other genotypes are negligible. The concurrent occurrence of HPV16 and HPV18 is found to be 1 in 15 cases of CIN. Almost two-fifths of the low-grade CINs and all of the high-grade CINs are observed to be positive for HPV. However, no significant association between HPV-DNA genotypes and CIN grade is evident. Available vaccine contains 16 and 18. In our geographical area 16 predominant and 18 is very less. Also other genotype has been detected in precancerous lesion. So we should suggest the policy makers to launch the vaccine after large scale study of HPV genotype in precancerous lesions. Accordingly vaccine should be prepared.

Recommendation

Based on the above conclusion; a large-scale multicenter study, including women from different geographic regions of Bangladesh, is recommended to estimate the true prevalence of HPV in women with CIN as well as its different genotypes. A follow-up study is also recommended to know which genotypes frequently contribute to the development of invasive cervical carcinoma or carcinoma in situ.

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