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Original Research Article

Efficacy of high-risk human papillomavirus genotype testing as cervical cancer screening method in a tertiary hospital of Dhaka, Bangladesh

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ABSTRACT

Background: The fourth most frequent gynecological malignancy worldwide is cervical cancer. Due to the high incidence of risk factors, cervical cancer is a pressing concern in Bangladesh. One of the most prevalent sexually transmitted viruses that can be chronic and can result in cervical cancer is the human papillomavirus (HPV). Investigating the epidemiology and clinical traits of this specific kind of HPV requires the identification of the high-risk (HR) HPV type. The aim of the study is to find out High risk HPV genotype (16, 18, and others) distribution among asymptomatic women and compare the diagnostic performance between the test of HR-HPV and visual inspection of cervix with acetic acid.
Methods: This cross sectional study was carried out in the Department of Gynecological Oncology, Bangabandhu Sheikh Mujib Medical University, Dhaka. A total of 300 asymptomatic women, aged 30 to 60 years, fulfilling inclusion criteria were included in this study. Study data was collected by a structured questionnaire designed for interview, clinical examination, HR-HPV genotyping, visual inspection of the cervix with acetic acid (VIA), and colposcopy of the women. HR-HPV genotyping was performed by a reverse transcriptase polymerase chain reaction.
Results: Among all of the HPV genotypes, HPV 16 (4.7%) was the most prevalent type, followed by HR-HPV (3.7%), HPV 18 (0.7%), and a combination of HPV 16 and other HR-HPV (0.3%).
Conclusions: HPV genotype can be used as an effective method for cervical cancer screening, including the identification of women at risk of cervical cancer.

Keywords: HPV genotype, VIA, HR-HPV, Preinvasive lesions, Invasive lesions

INTRODUCTION

Cervical cancer (CC) is the fourth most common cancer in women worldwide, accounting for 604,000 new cases and 34,000 deaths in 2020, according to World Health Organization (WHO) factsheets for February 2022. This is the second most common cancer among women in Bangladesh, with approximately 8,068 new cases detected every year and 5214 deaths.[1] A study by the National Strategy for Cervical Cancer Prevention and Control in Bangladesh (2017–2022) shows CC is a major concern in Bangladesh due to the high prevalence of risk factors such as early marriage, early sexual intercourse, multiparity, low socioeconomic status, and sexually transmitted diseases. Human papilloma virus (HPV) is the main causative agent of CC and is one of the most common viral infections of the genital tract. There are over 200 different HPV genotypes, and about 30 types of HPV infect the genital mucosa.[2] It has been categorized as low-risk HPV such as (6, 11, 42, 43, 44), intermediate-risk HPV such as (31, 33, 35, 51, 52), and high-risk HPV (16, 18, 45).[3] HPV viruses enter through a small break, such as during delivery in the epithelium near the squamo-columnar junction, and infect the cells of the basal layer of the squamous epithelium. Most of the women can clear the infection with their natural immunity, but a small number of women cannot clear the infection due to their compromised immune systems. Persistent HPV infection

causes neoplastic changes in the transformation zone, and persistent infection with HR-HPV is a critical event in the development of high-grade cervical lesions that can progress to invasive CC.[4] These viruses can be transmitted through genitalia, skin-to-skin, or skin-to-genitalia contact. The malignant process starts when the viral genome is integrated into the host genome. Moreover, the progression of HPV infection to invasive cancer is associated with some co-factors like multi-parity, age of first full-term pregnancy, and use of the oral contraceptive pill.[5] CC morbidity and mortality can be reduced through screening. There are various methods of CC screening, including cytology-based screening, visual inspection of the cervix after application of acetic acid (VIA), HPV DNA testing, and colposcopy. Among them, the HPV DNA test is the most reliable method because of its' high specificity and sensitivity. VIA is a simple and low-cost method, and the Government of Bangladesh has developed a VIA-based program to screen for cervical cancer, covering more than half the country. In low-socioeconomic countries, sensitivity and specificity of VIA are 93.6% and 58.3%, respectively.[6] Colposcopy, in conjunction with pre-cancer screening and treatment, played a significant role in lowering the incidence and mortality of CC.[7] During colposcopy, a binocular magnifying instrument examines the surface features and vascular pattern of the cervical and vaginal epithelium. Colposcopically directed biopsies can be taken from the most severely affected areas and sent for histopathological examination. It is essential to identify specific HPV types in order to investigate the epidemiology and clinical characteristics of these types. In several countries (such as the USA, Netherlands, and Italy), detection of specific HPV types in genital specimens has been approved in women with a cytological diagnosis of atypical squamous cell of undetermined significance and also for primary cervical screening as an adjunct to cytology in women aged 30 years and above.[8] To reduce the burden of pre-invasive and invasive diseases of the cervix, HPV genotyping improves the understanding of prevalence and the development of effective vaccines.[9] Identification of specific HPV genotypes helps in the selection of patients who are at increased risk of cervical disease. Hence, in order to know the specific genotype prevalence in the respective country, genotyping should be done because there is regional variation in HPV genotypes among the female population.[10] An effective HPV genotyping test will contribute to identify women at risk of developing CC and determine the specific clinical strategy.[11] There are two categories of HPV deoxyribonucleic acid (DNA) detection methods. One category uses the amplification of nucleic acids to detect the virus, known as the polymerase chain reaction (PCR) method. The other categories identify the nucleic acid directly and include Southern blot hybridization, dot blot hybridization, in situ hybridization, and hybrid capture liquid hybridization. The PCR can detect HPV types with known DNA sequences from a small amount of tissue. This is a highly sensitive method (sensitivity 94.7%), easy to interpret, and can characterize multiple virus types in the case of multiple infections.[12] For the diagnosis of HPV, the PCR technique is considered as the gold standard method.[13] Therefore, the present study aimed to detect high-risk HPV genotypes among sexually active women by PCR. The type-specific PCR detects target HPV DNA from different carcinogenic HPV types (high-risk genotypes 16, 18, and others). This study aims to compare the effectiveness of HR-HPV genotyping with VIA.

METHODS

This cross-sectional study was conducted in April 2021 to March 2022 in the department of gynecological oncology of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka. The study was approved by Bangabandhu Sheikh Mujib Medical University (BSMMU) Ethical Committee (BSMMU/2021/3167).

A total of 300 women aged between 30 to 60 years were selected for this study.

The inclusion criteria set for the women included in this study were as follows- married woman, 30 to 60 years of age, with no previous history of screening. The exclusion criteria set for the study were- patients who were pregnant or menstruating, had undergone partial or complete hysterectomy, women with known CC or history of taking any immunosuppressive drug or known case of immunosuppression were excluded from this research.

HPV genotyping report and VIA result were the main source of data. A semi-structured questionnaire was prepared for this purpose, which included all of the variables of interest. The participants were given a thorough explanation about the study objectives, rationale, and potential benefits and later written consent was obtained. Data was collected through interviews, physical examinations and laboratory investigations. The demographic information (age, occupation, and socio-economic condition) and previous medical records were collected on a pre-diagnosed data collection sheet. Study data was collected by face-to-face interview, privacy and confidentiality was ensured at all times during the process. All the other required data was collected from the patient's history report and investigation papers with their verbal consent for any information related to age of first delivery, admission and discharge dates. After examining the cervix for any suspected growth and discharge with a speculum, cervical specimens were collected by a cervix brush. During the process, the central bristle of a broom was inserted into the endocervical canal, which was deep enough to allow the shorter bristle to fully contact the ectocervix and rotated the broom clockwise direction five times. Then, the brush rinsed into the Cobas PCR cell collection media (Roche 2021. MC-CA-01790Laval, Québec Canada). by pushing the brush into the bottom of the vial ten times and discarded the head of the brush. Samples were stored at -200C at the PCR lab of the national screening center of BSMMU until analysis. HPV genotyping was performed by PCR (Cobas 4800 system,

Roche Diagnostics, GmbH, and Mannheim, Germany). Then 5% acetic acid was applied to the cervix and observed for 60 seconds to find the result which include the observation of aceto-white area if positive and if negative, no visible aceto-white area on the cervix. The colposcopy was done in the colposcopy clinic at BSMMU and biopsy was taken from the VIA positive patients of CC and sent for histopathology. The Cobas 4800 HPV test (Roche Diagnostics, GmbH, and Mannheim, Germany), a qualitative test device to detect HPV DNA, was used to analyze the samples. This test amplifies target DNA in cervical epithelial cells (Cobas PCR collection media, Roche Molecular Systems, Inc.) by PCR and nucleic acid hybridization to detect hr-HPV-16, 18 and other types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). Statistical analyses were carried out using the Statistical Package for Social Sciences version 23.0 for Windows (SPSS Inc., Chicago, Illinois, USA). For the validity of study outcome, sensitivity, specificity, accuracy, positive predictive value and negative predictive value of the VIA and HPV genotype diagnosis evaluation of cervical cancer were calculated. In the whole process, p values <0.05 was considered statistically significant.

RESULTS

This cross-sectional study was carried out in the department of gynecological oncology, BSMMU where 300 female patients who satisfied the inclusion criteria were selected for this study. Most of the participants (27.7%) were between the ages of 30 and 39 years. Mean age (for normal age distribution) of the sample was 40.0±7.1 with a range from 30 to 59 years. A total of 168 (56%) women completed secondary school certificate education, 200 (66.7%) were housewives, 282 (94.0%) were Muslim, and most of the women 172 (57.3%) were from a middle-income group (Table 1).

According to Table 2, the distribution of HR-HPV genotype among women of which 14 women (4.7%) are HPV 16 and 2 (0.7%) were HPV 18 positive. In Figure 1, 37 (12.3%) women were VIA positive and 263 (87.7%) were negative. Among 37 VIA positive women, 23 women were HR-HPV genotype positive (true positive) and 14 were negative (false positive). Among 263 VIA negative women 258 were HR-HPV genotype negative (true negative) (Table 3). The validity of CC screening by VIA was measured by calculating sensitivity, specificity, accuracy, positive and negative predictive values taken into account HR-HPV genotype. VIA was represented by calculating 82.1% sensitivity, 94.9% specificity, 93.7% accuracy, 62.2% PPV and 98.1% NPV (Table 4).

Among 37 VIA positive patients 26 women were colposcopy positive (true positive) and 11 were negative (false positive). Out of total of 263 VIA negative women 258were colposcopically negative (true negative) (Table 5). Among 28 HR-HPV positive patients 27 women were colposcopy positive (true positive), 1 was negative (false positive) and out of 272 HR-HPV negative women 268 were colposcopy negative (true negative) (Table 6). VIA was represented by calculating sensitivity was 83.9%, specificity was 95.9%, accuracy was 94.7%, PPV was70.3% and NPV was 98.1%. HR-HPV genotype was represented by calculating sensitivity was 87.1%, specificity was 99.6%, accuracy was 98.3%, PPV was 96.4% and NPV was 98.5% (Table 7).

Table 1: Distribution of the study population by socio-demographic characteristics (n=300).

Variables	Number of patients	Percentage
Age (years)		
30-34	83	27.70
35-39	81	27.00
40-44	58	19.30
45-49	36	12.00
50-54	31	10.30
55-59	11	3.70
Mean+SD	40.07.1	
Range (min-max)	30.0-59.0	
Educational status		
No schooling	51	17.00
Primary	81	27.00
SSC	82	27.30
HSC	60	20.00
Graduate and above	26	8.70
Occupational status		
Housewife	200	66.70
Service holder	50	16.70
Others	50	16.70
Religion		
Muslim	282	94
Hindu	18	6
Monthly family income (Taka)		
Low income group (<6827 BDT)	128	42.7
Middle income group (6828- 26852 BDT)	172	57.3

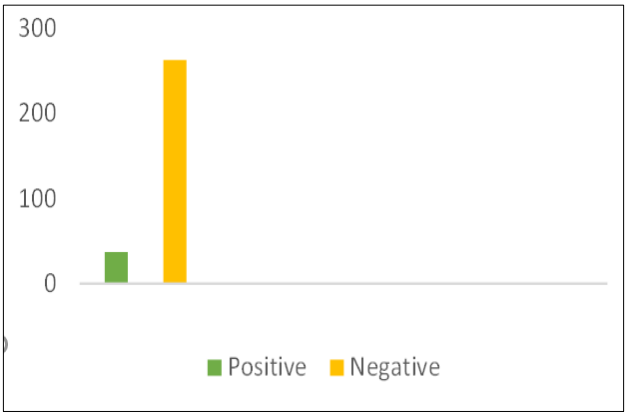

Figure 1: VIA finding (n=300).

Table 2: HR-HPV genotype of the study population (n=300).

Type of HR-HPV genotype	Number of patients	Percentage
HPV 16	14	4.7
HPV 18	2	0.7
Other HR-HPV	11	3.7
HPV 16 and other HR-HPV	1	0.3
Negative for HR-HPV	272	90.7

Table 3: Comparison between VIA and HR-HPV (n=300).

VIA findings	HR-HPV	
	Positive (n=28)	**Negative (n=272)**
Positive (n=37)	23 (true positive)	14 (false positive)
Negative (n=263)	5 (false negative)	258 (true negative)

Table 4: Comparison of sensitivity, specificity, accuracy, positive and negative predictive values.

Diagnostic accuracy	VIA	95% CI (lower-upper)
Sensitivity	82.1	63.11-93.94
Specificity	94.9	91.51-97.16
Accuracy	93.7	90.29-96.14
Positive predictive value	62.2	48.95-73.79
Negative predictive value	98.1	95.89-99.13

Table 5: Comparison between colposcopy and VIA findings (n=300).

VIA findings	Colposcopy findings	
	Positive (n=31)	**Negative (n=269)**
Positive (n=37)	26 (true positive)	11 (false positive)
Negative (n=263)	5 (false negative)	258 (true negative)

Table 6: Comparison between colposcopy and hr-HPV genotype findings (n=300).

HR-HPV genotype	Colposcopy findings	
	Positive (n=31)	**Negative (n=269)**
Positive (n=28)	27 (true positive)	1 (false positive)
Negative (n=272)	4 (false negative)	268 (true negative)

Table 7: Diagnostic performance test of VIA and HR-HPV genotype.

Diagnostic accuracy	Sensitivity	Specificity	Accuracy	PPV	NPV
VIA	83.9	95.9	94.7	70.3	98.1
HR-HPV genotype	87.1	99.6	98.3	96.4	98.5
P value	0.722	0.004	0.017	0.009	0.720

DISCUSSION

The aim of the study was to compare the effectiveness between HR-HPV and VIA. There is scarcity of information on HPV cervical infection in Bangladesh. Moreover, clinical data for primary HPV screening alone is currently lacking. Our aim was to investigate the HR-HPV distribution among asymptomatic women in Bangladesh. The detection rate of cervical precancer and cancer by VIA and HR-HPV genotype is reflected in this study by calculating sensitivity, specificity, accuracy, positive and negative predictive values using colposcopy as the gold standard. In our study, HR-HPV genotyping has shown to have a higher sensitivity and specificity than VIA as a screening method. A sensitivity of 83.9%, specificity of 95.9%, accuracy of 94.7%, PPV of 70.3%, and NPV of 98.1% were estimated for the VIA. In a different comparison study conducted in Nigeria, the VIA's sensitivity was 60%, specificity was 94.4%, the PPV was 50%, and the NPV was 99.4%.[14] However, multiple investigations indicated that VIA's sensitivity was greater than its specificity. which is in opposition to our study. As a result of the researcher alone collecting, analyzing, and interpreting the sample, there was less inter-observer variation in this study, and we observed that VIA's specificity was greater than its sensitivity. A hospital-based study done in Bangladesh (in 2010) found that VIA sensitivity in 2188 patients was 93.6%.[15] Because of inter-observer variances and a lack of a consistent, reproducible system for classifying and reporting VIA, the results of different studies on VIA varied. When calculating HR-HPV genotype, sensitivity was 87.1%, specificity was 99.6 %, PPV 96.4% and NPV 98.5%. However, in this investigation, the HR-HPV genotype had a higher sensitivity and specificity. The findings of this study are in line with those of a population-based study conducted in India, a neighboring nation to our study area, which found that HPV testing showed higher sensitivity (100%) and specificity (90.6%) than VIA.[16]

According to WHO objectives, CC can be eradicated by 2030 by the implementation of a global strategy involving the vaccination of young girls against HPV, screening 70% of women in 30–69 years of age and treating 90% of the women with precancerous lesions.[17] For a country with a large population like Bangladesh, implementation of the three strategies can be a challenge. There is a need for implementation of a high throughput technology that can

be scalable. Cobas 4800, a multiplexed assay based on quantitative polymerase chain reaction technology, identifies HPV 16 and HPV 18 along with the concurrent detection of 12 pooled other HR-HPV infections. This technology was used to test 10 375 women from the South Indian community for the first time as a feasibility program.[18] Upon testing, HR- HPV was found in 595 (5.73%) women where a total of 127 women (1.2%) were found to be infected with HPV 16, 36 women (0.34%) with HPV 18 and 382 women (3.68%) with the 12 pooled HR-HPV and multiple mixed infections were found in 50 women (0.48%). It was observed that there was a high prevalence of HR-HPV in young women aged 30–40 years and a second peak was observed at 46–50 years of age. The second peak had higher mixed infections in the 46–50 years of age and this association was statistically significant.[1] It was also found in the same research that 24/50 (48%) of the multiple mixed high-risk HPV infections were in the age group 46–50 years, which is quite similar to this current study age range. A study, as the first attempt from India, on a completely automated platform used Cobas 4800 HPV test in a community screening program showed HPV 16 and HPV 18 infections, when differentiated, can be valuable for risk stratification in community screening program.[1] Women in the perimenopausal age (46-50 years) showed a higher prevalence of multiple mixed infections, signifying a higher risk.[18]

The essential objective of CC screening is to decrease mortality by distinguishing precancerous and cancerous lesions and mediating to prevent the progression to CC. It is essential to perceive that the risk of HPV disease is most elevated among the individuals who are recently sexually active, and that the risk decreases with age. The pinnacle rate of high-risk HPV disease is in youngsters and in ladies in their mid-20s. The movement from persevering high-risk HPV contamination to intrusive cervical infection takes a normal of 10 to 20 years. Due to this slow oncogenesis, persevering HR-HPV infections that manifest as irregularities of the cervix can be recognized early, bringing about less-intrusive treatments and subsequently reduced adverse outcomes.[19] Larger part of the female population (69.5%) came to be aware of the VIA test from gynecology oncology specialists as specialists are accepting the open-door approach in order to allude patients to these effectively accessible administrations. In any case, this emergency clinic-based setting gives data from women referred principally by doctors in an urban setting. Then again, population-based study from rural areas of Bangladesh observed that around 1/10th of provincial females knew about 'VIA' where facility of VIA was available, and only about 1% knew about VIA where facility of VIA was unavailable. According to this study, accessibility of screening administrations impacts mindfulness level about screening among individuals.[20] HR-HPV infection is the leading source of CC, especially HPV-16 and HPV-18 which represent almost 70% of every single cervical disease. Before 2018, algorithms for both co-testing and essential HPV screening rules depended exclusively on fractional genotyping and didn't reflect the distinction in risk for CIN 2 or more hazardous (≥CIN 2) or aggressive CIN 3 and more, among ladies with different non-16/18 HPV genotypes. In a systematic review, evidence is introduced that depicts appraisal of oncogenic risk for individual HPV genotypes with regards to cervical malignant growth screening.[21]

In this study it is found, among 300 women 9.3% women were HR-HPV positive. In Dhaka, division of Bangladesh, prevalence of HPV infection was 7.7% with no significant difference between urban and rural area.[22] A study in China revealed HPV positivity was 8.16% among women of Shahn City. Another study in China showed 12% women tested positive for HPV23. In Maharashtra, it was found that prevalence of HPV infection was 10.3% among 30-59 years of age.[24] A study conducted in 2012, showed that prevalence of HPV infection was 9.9% in Eastern India.[25] In a hospital-based study prevalence of HR-HPV was found to be 10% by PCR.[26]

Therefore, HPV prevalence of Bangladesh is similar to China and India. The prevalence of HPV varies from 24-38.5% in African countries which is much higher compare to Bangladesh.[18.27]

Limitations

Our study was a single center study. We could only study a few samples within the study period where number of female under the threat of CC is vast. There are more patients with CC possibilities which is affecting their life but they are not coming to the hospital for proper treatment because of the taboo attached with reproductive health of women. In some remote areas, female patients are highly conscious of testing because of their religious views which is resulting in less data collection for research purposes and also, increases the probability of not getting proper diagnosis; consequently, risking their lives.

CONCLUSION

In light of the consequences of this study and their correlation with different samples, it can be concluded that hr-HPV genotyping is more effective and less risking than VIA. It is comparatively more effective and easier screening system that helps to identify and take initial steps to prevent risk of cervical cancer.

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