Frequency and Genotyping of High Risk Human Papilloma Virus among VIA Positive Women Attending at Colposcopy Clinic, Chittagong Medical College Hospital

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Abstract

Background: Worldwide, cervical cancer is one of the most important cancers in women, being responsible for 569,847 new cases and 311,365 related deaths in 2018, according to GLOBOCAN. There are several methods to recognize HPV. Among them, PCR has been widely used to detect HPV DNA. Purpose of this study was to determine the frequency and genotyping of the high risk Human Papilloma Virus (16, 18) among VIA positive women attending colposcopy clinic.

Materials and methods: This was a cross sectional study and was conducted in the Colposcopy Clinic, Department of Obstetrics and Gynecology of Chittagong Medical College Hospital from January 2021 to June 2021. During the study period 50 VIA positive women attending colposcopy clinic were taken as study subjects. Data were collected by a predesigned case record form. Analysis was done using computer software SPSS version 25.

Results: Out of 50 VIA positive women, the mean age was 37.14 ± 4.5 years. Colposcopy report of VIA positive patients shows that 41 (82%) had normal, 8 (16%) CIN I and 1 (2%) CIN III. PCR analysis shows that 8 (16%) positive. Molecular genotyping of PCR positive patients shows that 5 (62.5%) were infected by HPV 16 and 3 (37.5%) were infected by HPV 18. There was no significant difference between colposcopy findings and PCR findings. Age at marriage, age at full term pregnancy, H/O husband living abroad and husband having promiscuous relationship showed significantly greater chance of having positive result by PCR analysis.

Conclusion: Age at marriage, age at first pregnancy, H/O husband living abroad and husband having promiscuous relationship showed greater chance of having HPV infection. Early detection of HPV and early diagnosis of precancerous lesions can lower the chance of cervical cancer.

Kew words: Colposcopy; Papilloma Virus; VIA.

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Introduction

Cervical carcinoma is the second most important cause of cancer induced deaths in females of Bangladesh and has annual incidence of about 11,956.[1] The prevalence of cervical cancer in Bangladeshi women has been reported to be 25–30/100000.[2] There were an estimated 527,600 new cervical cancer cases and 265,700 deaths worldwide.[3]

This high prevalence of cervical cancer in developing countries is related to many risk factors such as early marriage, early starting of sexual activity, multiparity, low socioeconomic condition and high incidence of Sexually Transmitted Diseases and Human Papilloma Virus (HPV) infection.[4]

Several studies established HPV infection as a sexually transmitted disease and major risk factor for development of Cervical Intraepithelial Neoplasia (CIN) and Invasive Cervical Cancer (ICC).[5-6] Research also showed that over 90% of cervical cancers worldwide contained HPV DNA.[3]

HPV vaccination, on the contrary, provides an opportunity to low-resource settings, such as Bangladesh, to reduce the burden of cervical cancer through primary prevention and successful HPV vaccination uptake among the target population of adolescents.[7]

The advent of HPV testing has opened the doors for more accurate cervical cancer screening strategies. There are several methods to recognize HPV associated disease such as cytological evaluation, colposcopy, biopsy, tissue diagnosis test and molecular methods such as Southern Blot, Dot Blot Hybridization, PCR and so on.[8,9]

Among them, PCR has been widely used to detect HPV DNA and it's a laboratory technique. The purpose of PCR testing is to find small amounts of DNA in a sample using a process known as amplification.[10]

The use of acetic acid during visual examination of the cervix, termed visual inspection with acetic acid (VIA), has been advocated as an alternative screening method to Pap smear in developing countries.[11] The attractive features of VIA include low cost, simple administration, real-time screening, of results, and accuracy comparable to good quality Pap smears.[12] In a developing nation like Bangladesh VIA would be a possible alternative screening tool for early detection of cervical cancer in a low resource setting. VIA is not recommended for women over age 50 years.[13]

A colposcopy is a special way of looking at the cervix. It uses a light and a low-powered microscope to make the cervix appear much larger. Colposcopy is a worldwide-accepted method for detection of early cervical neoplasia.[14] Common problem encountered in colposcopy is inadequate expertise, interpretation difficulties, disagreements and failure to follow standard diagnostic protocol.[15] So it is better to do colposcopy in all VIA positive patients rather as a screening procedure.

HPV DNA testing by PCR can be done easily. Early diagnosis of CIN would help in reducing invasive cancer and therapy rendering effective and curative treatment for pre-invasive disease. Therefore in this study, we evaluate the frequency and genotyping of high risk HPV among VIA positive women.

Materials and methods

This descriptivetype of cross sectional study was conducted in the Colposcopy clinic, Department of Obstetrics and Gynecology, Chittagong Medical College Hospital (CMCH) Chattagram during the period of January 2021 to June 2021. Before starting this study ethical clearance was taken from Institutional Review Board (IRB) of CMCH. A total of 50 VIA positive women attending colposcopy clinic at CMCH and gave informed written consent for the study were included in the study. Patients with cervical growth, active vaginal bleeding, patients using vaginal suppository within 14 days, undergone any vaginal / cervical procedure within 14 days and patients unwilling to participate in the study were excluded from the study.

Specimen was collected with the Digene Hybrid Capture 2 (HC2) DNA collection device which contains 1 Brush and 1 ml specimen transport medium (contain 0.05% sodium azide). At first excess mucous was removed from the cervical os and surrounding ectocervix using a cotton swab. Then the Brush was inserted 1- 1.5cm in to the os of the cervix until the largest outer bristles of the brush touch the ectocervix. It was rotated 3 full turns in a counter clock wise direction. Then brush was removed from the canal. Then the brush was inserted to the bottom of the transport tube. At first specimen was collected and then colposcopy was done in the same setting.Collected specimens can be stored at 2-8℃ for 7 days or -20℃ for 3 months prior to testing.

The diagnostic kit uses a nucleic acid lysis buffer to allow rapid lysis and release of HPV-DNA. By applying real-time fluorescence quantitative PCR technology, this test uses a set of specific primers and specific fluorescence probes which are designed to target a conserved sequence of HPV16 DNA and HPV18 DNA, accompanied with other ingredients in PCR mix, to achieve fast detection of HPVDNA and also HPV16 and HPV18 virus samples through fluorescent signal changes. The PCR detection system uses UNG enzyme + dUTPcontaminationproof system to fully degrade possible unwanted side-products in order to avoid a false positive result.

Data was entered, cleaned and analyzed using Statistical Package for Social Sciences (SPSS -25) software. Continuous variables were statistically described in terms of mean and standard deviations ($\pm$ SD). Qualitative or categorical variables were described as frequencies and proportions. Unpaired t-test was used for continuous variables and McNemar testand Chi square test was used for categorical variables.Statistical significance was defined as p $\leq$ 0.05.

Results

Out of 50 patients, mean $\pm$ SD age of VIA positive woman was 37.14±4.5 years,para was 3-4 in most of the patients (52%). Maximum (84%) patients had history of multiple pregnancy. Mean±SD age of marriage was 17.08 $\pm$ 2.4 years and age of full term pregnancy was 18.92 $\pm$ 2.3 years (Table I). Colposcopy report of VIA positive patients, 41 (82%)

had normal, 8 (16%) had CIN I and 1 (2%) had CIN III (Figure 1). PCR analysis of VIA positive, 8 (16%) positive and 42(84%) had negative report (Figure-2). Molecular genotyping of PCR positive patients, 5 (62.5%) were infected by HPV 16 and 3(37.5%) were infected by HPV 18 (Figure-3). There was no significant difference between colposcopy findings and PCR findings (p=0.100) (Table II). The association of age at marriage, age at full term pregnancy, H/O husband living abroad and husband having promiscuous relationship showed greater chance of having positive result by PCR analysis (Statistically significant according to p value) (Table III).

Table I Socio-demographic characteristics and obstetrics profile of the patients (n=50)

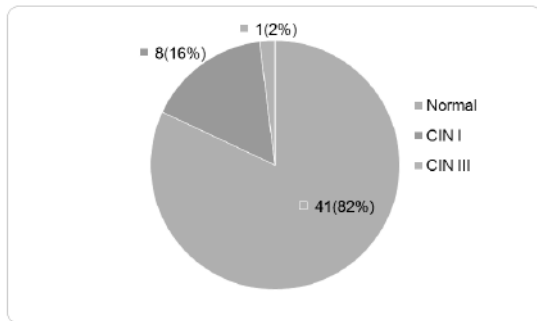| Characteristics			
Age (Years)	Mean±SD	37.14±4.445	
		Frequency	Percentage
Monthlyfamilyincome	Low	4	8%
	Lowermiddle	43	86%
	Uppermiddle	3	6%
Para	≤ 2	8	16%
	3-4	26	52%
	≥ 5	16	32%
Having multiple pregnancy	No	8	16%
	Yes	42	84%
Age at marriage (Years)	Mean±SD	17.08±2.415	
Age ate fullterm pregnancy (Years)	Mean±SD	18.92±2.346	

Figure 1 Colposcopy report among the patients (n=50)
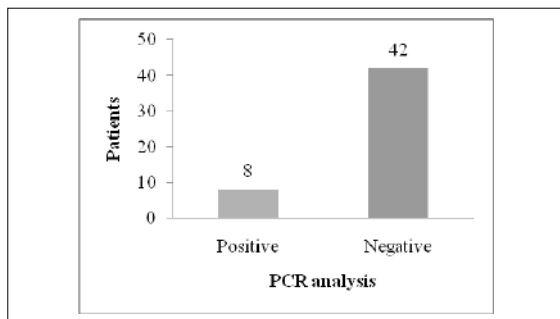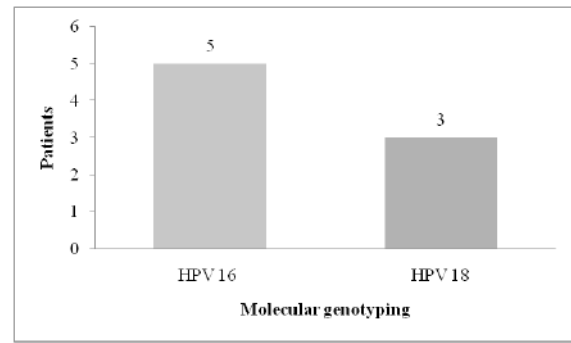

Figure 2 PCR analysis of the patients (n= 50)

Figure 3 Molecular genotyping of the PCR positive patients (n=8)

Table II Comparison between PCR analysis and colposcopy report (n=50)

PCR		Colposcopy report				p value
		Positive (n=9)		Negative (n=41)		
		n	%	n	%	
Positive		8	88.9	0	0.0	1.00
Negative		1	11.1	41	100.0	

Table III Association of factors with PCR analysis

Variables		PCR analysis		p value
		Positive (n=8)	Negative (n=42)	
Age (Years)	Mean±SD	35.92±2.3	37.28±4.5	0.411# (ns)
Age at marriage (Years)	Mean±SD	14.42±0.5	17.36±2.3	<0.001# (hs)
Age at full term pregnancy (Years)	Mean±SD	16.21±0.4	19.25±2.2	<0.001# (hs)
Husband living abroad				
	Yes	3 (37.5%)	2 (4.8%)	0.024* (s)
	No	5 (62.5%)	40(95.2%)	
Husband having promiscuous relationship				
	Yes	2 (25%)	0(0.0%)	0.023* (s)
	No	6 (75%)	42 (100%)	

p value derived from Student "t" test (#) and Chi-square test (*), ns= non-significant, s= significant, hs= highly significant

Discussion

Cervical cancer continues to be a major problem in Bangladesh with approximately18,000 new cases annually of which over 10,000 women die from it.[16]High-risk Human Papilloma Virus (HPV) is found in over 99% of cervical cancers.[17]

In this present study, mean ± SD age of VIA positive woman was 37.14 ± 4.5 years. A recent study conducted by Sousa et al. stated that a total of 105,458 women (Mean age 43.8 ± 10.6 years old.[18] Another recent study by Aguilar et al. described the mean age was 40.7±13.5 years.[19]

Present study shows, out of 50 patient's colposcopy report of VIA positive patients. The findings shows that 41 (82%) had normal, 8 (16%) had CIN Iand 1 (2%) had CINIII. widerska-Kiec et al. reported that a total of 101 patients (54%) had negative colposcopy results, whereas 59 patients (32%) had suspicious results and 26 women (14%) had colposcopy results which were deemed unsatisfactory.[20]

Concerning PCR analysis of VIA positive patients shows that 8 (16%) positive and 42(84%) had negative report. In the study by Akcali et al. showed positive HPV was detected in 35 out of 410 patients (8.5%) by PCR analysis.[21]

Regarding molecular genotyping of PCR positive patients. It shows that 5 (62.5%) wereinfected by HPV 16 and 3 (37.5%) were infected by HPV 18. A study by Traore et al. reported that, HPV 18 was 14.8% but HPV 16 was not found in the women included in their study.[17] Sousa et al. found, HPV-16 in 17.5% and HPV-18 in 5.12% patients.[18] Another study by Nahar etal. revealed, HPV 16 was in 23 and HPV 18 in 4 cases.[22]

In this study observed that colposcopy positive was found in 9 cases, among them 8(88.9%) cases were positive and 1(11.1%) was negative confirmed by PCR. Colposcopy negative was 41 cases and all were negative confirmed by PCR, that was not significant (p=1.00). widerska-Kiec et al. had observed that the HPV test is more sensitive than colposcopy for detecting CIN1+ cases, with values of 79.4% for the HPV test and 73.7% for colposcopy.[20] When comparing each test completed alone, the PPV was higher for the colposcopy examination (71.2%) compared to the HPV test (50.5%) in detecting any dysplasia (CIN1+). in a study from Germany, in which the PPV for the HPV test was 36%, it was 38% for colposcopy.[23]

In this study showed age at marriage, age at first full term pregnancy, H/O husband living abroad and husband having promiscuous relationship showed greater chance of having positive result by PCR analysis (Statistically significant according to p value). widerska-Kiec et al. described that HPV infections were most common in women in age group 18-29 years, in whom the virus was detected in 76.2% of the patients. The lowest number of infections (25% of patients) was observed in the group aged over 60 years.[20]

Limitation
The study population was selected from one selected hospital in Chattogram city, so that the results of the study may not reflect the exact picture of the country, avery short period of time and small sample size.

Conclusion
Colposcopy positive was 18.0% and PCR positive in 16.0% patients. There was no significant difference between colposcopy findings and PCR findings among VIA positive patients. However, age at marriage, age at full term pregnancy, H/O husband living abroad and husband having promiscuous relationship showed greater chance of having HPV infection. Early detection of HPV and early diagnosis of precancerous lesions can lower the chance of cervical cancer.

Recommendation
HPV screening would thus allow follow-up efforts to be targeted to women at greatest risk for the disease. Cost-effectiveness analysis of HPV DNA testing is urgently needed to evaluate its application in various health care settings.

Contribution of authors
DS-Conception, design, critical revision, acquisition of data, data analysis, manuscript writing & final approval.
SC-Conception, critical revision & final approval.
FIC-Design, interpretation of data, critical revision & final approval.
SM-Design, drafting & final approval.
FA-Design, interpretation of data, drafting & final approval.
TT-Acquisition of data, drafting & final approval.

Disclosure
All the authors declared no competing interest.

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