Original Article

Human papillomavirus infection among Bangladeshi women with cervical intraepithelial neoplasia and chronic cervicitis

Elisha Khandker[1], Mansura Khan[2], Ahesh Kumar Chowdhury[2]

[1]Department of Microbiology, Ibrahim Medical College, [2]Department of Immunology, Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders, Dhaka

Abstract

Background and objectives: Cervical cancer is one of the leading causes of morbidity and mortality. Human papillomavirus (HPV) is known to be associated with cervical intraepithelial neoplasia (CIN) and cancer. The objective of the present study was to determine the rate of HPV infection among the Bangladeshi women with different grades of CIN and cancer.

Methods: Women aged 20 to 55 years, diagnosed as a case of chronic cervicits, cervical intraepithelial neoplasia (CIN) or invasive cancer by Papanicolaou (Pap) smear and colposcopy directed biopsy were enrolled in the study. High and intermediate risk oncogenic HPV were detected in cervical samples by real time PCR (rt-PCR).

Results: Seventy two women with chronic cervicitis and different grades of CIN were included in the study. Out of 72 cases, 28 (38.9%) and 44 (61.1%) had chronic cervicitis and CIN respectively. Overall, the HPV infection rate was 43.1% (95% CI= 32%-54%) among the study population. CIN cases had significantly high ($p<0.01$) HPV infection (78.6%; 95% CI=60%-89%) compared to cases with chronic cervicitis (18.2%; 95% CI=11.1%-34.5%). Women between the age of 20-30 years had the highest positive rate (50.0%) followed by 31-40 years age group (43.6%). All CIN grade 2 and 3 had HPV infection.

Conclusion: The study showed that HPV was strongly associated with different grades of CIN. Specific HPV types should be determined to find out the most prevalent HPV types among the Bangladeshi women with CIN and cervical cancers.

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Introduction

Cervical cancer is the fourth most common cancer in women in the world [1]. In Bangladesh, each year an estimated thirteen thousand women are diagnosed with cervical cancer and about six thousand die from the disease [2]. Persistent infection with oncogenic HPV is known to be associated with the development of cervical intraepithelial neoplasia (CIN) and cancer [3]. Globally, the prevalence of HPV infection is 11.7% among women without abnormal cervical cytology [4,5] while the rate is much higher among women with cervical dysplasia and cancer. The prevalence rate of HPV infection in different countries ranged from 1.6% to 41.9% [5]. A meta analysis hasreported an overall rate of HPV infection among African women with normal cervical cytology as 29.0% while the range varied from 12% to as high as 76.3% [6,7]. The prevalence of HPV infection in women with normal cervical cytology ranged from 6.7% to 11.4% in China, Nepal, Thailand and Indonesia [8-11]. A community based study in Bangladesh has reported a prevalence of HPV infection as 7.7% in women with normal cytology [12] while the overall rate is

Address for Correspondence:
Dr. Elisha Khandker, Department of Microbiology, Ibrahim Medical College, 122 Kazi Nazrul Islam Avenue, Shahbag, Dhaka 1000, Bangladesh. Email: elishakhandk@ymail.com

49.1% among the Bangladeshi sex workers [13]. There is little information regarding the prevalence of HPV infection in Bangladeshi women with cervical dysplasia and cancer. In 2009, a study from Bangladesh has reported 7-10% HPV infection in women with CIN and cervical cancer [14]. Therefore, the present study was undertaken to determine the prevalence rate of HPV infection among the women with CIN and cervical cancer.

Materials and methods

The study protocol was approved by the Ethical Review Committee of the Diabetic Association of Bangladesh. Informed consent was obtained from each participant.

Study population and place: This cross sectional observational study was carried out at Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM) and a private clinic of Dhaka city from July 2012 to June 2013. Women aged 20 to 55 years, diagnosed as a case of chronic cervicitis, cervical intraepithelial neoplasia (CIN) or invasive cancer by Papanicolaou (Pap) smear and colposcopy directed biopsy were enrolled in the study.

Collection of samples for detection of HPV: The cervical specimen for the detection of HPV by rt-PCR was collected with cervical brush provided with the commercial kit. Briefly, excess mucus from the cervical *os* and surrounding ectocervix was removed by a cotton swab. The sampling brush was then inserted into the cervical *os* until the largest bristle touched the ectocervix. The brush was rotated three full turns counterclockwise and removed and immediately placed in the 0.3 ml transport media. The container was shaken vigorously for 15-20 seconds and stored at -80^{0}C until processed.

Detection of HPV by real time PCR: HPV High Risk Screen Real-TM Quant 2x commercial kit (Sacace, Biotechnoloies Srl, Italy) was used for the extraction of DNA and detection of HPV by real time PCR. DNA was extracted and specific E1-E2 region of 12 HPV genotypes was amplified by real time PCR (Smart Cyler, Cepheid) according to the instruction of the manufacturer. The kit detected E1-E2 region of four high risk (16, 18, 31 and 45) and eight intermediate risk (33, 35, 39, 51, 52, 56, 58 and 59) HPV types. Any sample giving a Ct value $\leq$33 or HPV DNA concentration of $>$log3 was considered positive. The kit did not differentiate the individual genotypes.

Results

A total of 72 women with chronic cervicitis and different grades of CIN were included in the study. Out of 72 cases, 28 (38.9%) and 44 (61.1%) had chronic cervicitis and CIN respectively. The distribution of HPV positive cases in different age groups is shown in Table-1. Overall, the HPV positive rate was 43.1% among the study population. Women between the age of 20-30 years had the highest positive rate (50.0%) followed by 31-40 years age group (43.6%). Age group 20-30 and 31-40 years had significantly higher ($p<0.05$) HPV positive rate compared to women above 40 years of age. Table 2 shows that cases with CIN had significantly high ($p<0.01$) HPV infection (78.6%) compared to those with chronic cervicitis (18.2%). All cases of CIN 2 and CIN 3 were positive for HPV compared to 57.1% cases of CIN 1.

Table-1: *Distribution of HPV in different age groups*

Age group (years)	Total Case N	Total positive N (%)
20-30	26	13 (50.0)
31 – 40	32	14 (43.6)
$>$40	14	4 (28.1)
Total	72	31 (43.1)*

Note: *$p<0.05$ when compared among the groups.*
**95% CI for total HPV positivity rate= 32%-54%*

Table-2: *HPV infection in study population with different grade of CIN and chronic cervicitis*

Category	Number of case	HPV positive case No (%)
1. Chronic cervicitis	44	9 (20.5)
2. Total CIN	28	22 (78.6)
CIN 1	14	09 (57.1)
CIN 2	11	11 (100.0)
CIN 3	3	3 (100.0)

Note: $p < 0.01$, compared between the CIN and chronic cervicitis group.
95% CI for CIN= 60%-89%; Chronic cervicitis= 11.1%-34.5%

Discussion

The present study has demonstrated that high and intermediate risk oncogenic HPV types are prevalent in different grades of CIN and chronic cervicitis cases. All CIN grade 2 and 3 cases were positive for HPV while >50% was positive in CIN1. The rate of HPV infection was significantly low in chronic cervicitis (18.2%) but it was comparatively higher than that in women with normal cervical cytology [12]. Studies in different countries of Africa reported similar high rate of HPV infection in different grade of CIN [6]. But a previous study from Bangladesh in 2009 reported only 7-10% HPV positivity rate among CIN 2, CIN 3 and invasive carcinoma of the cervix [14]. In the present study, we could not determine the prevalence of particular HPV types as the commercial kit we used detected both high and intermediate risk HPV types as a whole. A previous study in Bangladesh has reported the presence of high risk HPV type (16, 18, 31 and 45) in about 56% of women with high risk behavior [13]. Similarly, a population based study in Bangladesh also found the prevalence of high risk HPV types among women with normal cytology [12].

In this study, there was increased positivity (50%) of HPV among the women of 20-40 years i.e. the reproductive age group women. The number was less in the extreme of age (>40 years). It was probably that younger CIN cases had infection with high risk oncogenic HPV types in their early life which progressed into CIN in middle age. The higher rate of HPV infection among women of this age group was found in other studies [6,13].

Therefore, it is important to determine the types of HPV in CIN and cervical cancer in larger patient group to have the maximum benefit from vaccination against HPV infection.

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