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Detection and estimation of human papillomavirus viral load in patients with cervical lesions

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Abstract

Human papillomavirus (HPV) high risk genotype infection and HPV viral load influences the development of invasive cervical cancer and cervical intra-epithelial neoplasia (CIN). HPV DNA testing for screening of cervical cancers may play a potential role in its early detection and management. The present study detected HPV DNA and estimated HPV viral load in different types of cervical lesions among Bangladeshi women. Using the Hybrid Capture 2 (HC2) assay, HPV DNA was tested among 68 women between 25-70 years of age. A total of 13 (19.1%) cases were positive for HPV DNA. The highest viral load (501 x 10^3 copies/ml) was detected in a patient with invasive carcinoma, while the lowest viral load (105 x 10^3 copies/ml) was detected from a case of chronic cervicitis. The mean viral load in CIN I was 119.25 x 10^3±12.5 x 10^3 copies/ml (range: 110 x 10^3 - 137 x 10^3) and 208.50 x 10^3 ± 0.59 x 10^3 copies/ml (range: 139 x 10^3-305 x 10^3) in CIN II / III. Interestingly, HPV DNA was detected from a patient with normal cytological findings. Our study observed a moderate presence of high-risk HPV genotypes among women with cervical lesions. The HPV viral load varied with the age of the patients and stage of cervical lesions. The HC2 assay is a promising tool for diagnosing high-risk HPV infection especially before cytology tests show any abnormality.

Introduction

Worldwide, cervical carcinoma ranks second among the common cancers in women, with an estimated 4,70,600 new cases and 2,33,400 deaths per year[1-2]. Cervical cancer primarily occur in developing countries where women have limited or no access to effective disease prevention services. Among the Asian countries, India, Bangladesh, Nepal and Sri Lanka together contribute to around one-third of the global cervical cancer burden[3]. In South Asia, Bangladesh and India have an annual incidence of 11,956 and 1,25,952 respectively[4].

The human papillomavirus (HPV), an oncogenic DNA virus and a member of the papovoviridae family is the main cause of most cervical cancers and cervical intraepithelial neoplasias (CIN) worldwide[1]. The virus is predominantly sexually transmitted and is a high–risk factor for development of cervical carcinoma[5-7]. The prevalence of human papillomavirus infection varies from 7 - 14% among the general population in India, Bangladesh, Nepal and Sri Lanka[8]. Persistent infection with certain genotypes of carcinogenic HPV causes most cases of cervical cancers[9]. Among 130 genotypes of HPV, types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 are "high risk" HPVs. Globally, HPV 16 and 18 contribute to over 70% of all cervical cancers while HPV types 31, 33, 35, 45, 52 and 58 are responsible for an additional 20% of cases[10]. The effect of high-risk types of HPV infection on CIN development is greatly influenced by the number of viral copies, as higher loads are more strongly associated with severe disease. Various studies suggest that a high HPV-DNA viral load may be a potential marker for identifying women at greater risk of CIN progression, while some indicate that high viral load in cytological normal epithelium may also be a risk factor for neoplastic progression[11-13].

As cervical cancer generally develops slowly from precursor lesions, regular screening for high risk HPV types should be performed to identify lesions with potential for progression to invasive cancers[14]. At present, molecular detection of HPV DNA is the gold standard for identification of HPV, and is being evaluated as a prospective alternative or adjunctive to cervical cytology for the early detection of cervical cancer precursors and prevention of invasive cervical cancer[15]. Information on HPV type-specific prevalence or viral load in women with or without cervical lesions is very limited from Bangladesh. Therefore, the present study detected HPV DNA and estimated

the viral load by the Hybrid capture 2 assay to determine the efficacy of this test among patients with different grades of cervical diseases.

Materials and Methods

The study was conducted among 68 women with different types of cervical lesions from January to December, 2008. Patients were selected from the Obstetrics and Gynecology Out-Patients Department (OPD) of Bangabandhu Sheikh Mujib Medical University (BSMMU) Hospital, Dhaka. Conventional methods are used for diagnosis of cervical lesion of the patients included Visible Inspection of Acetic acid (VIA), colposcopy, histopathology and pap smear tests. Sexually active women above 25 years of age having history of post-coital bleeding, per-vaginal spotting and /or spontaneous bleeding, patients referred for colposcopy due to abnormalities detected on previous pap's smears, VIA test and histopathological examinations, clinically unhealthy looking cervix on per-vaginal examination, patients with low-grade squamous intra-epithelial lesions (LSIL) included in this study.

After taking informed written consent, cervical specimen for HPV DNA was collected in a cervical sampler consisting of a cervical brush and specimen transport medium (STM), supplied by the manufacturer of HC2 (Digene Corporation, USA). Detection of HPV and estimation of viral load was carried out at the Department of Virology, BSMMU.

At the Molecular Virology Laboratory of the Dept. of Virology BSMMU, specimens were stored at –20° C until tested. Determination of HPV and estimation of viral load was done by the hybrid capture 2 (HC2) high-risk HPV DNA test kit (Digene Corporation, Gaithersburg, MD 20878, USA; catalog no-21293) according to the manufacturer's instructions. It detected 13 high-risk HPV types ie, 16, 18, 31, 33, 35, 45, 52, 56, 58, 59 and 68. Briefly, specimens were treated with sodium hydroxide to release and denature the DNA from cervical cells and hybridized to RNA probes designed for high-risk HPV types. The DNA-RNA hybrids that formed were captured onto the surface of microtiter wells using antibodies specifically designed for hybrid molecules. Then, alkaline phosphatase was added. Unreacted material was removed by washing, and chemiluminescent substrate (dioxetane) was added as a substrate. The fluorescent activity was measured using a luminometer and result of the test was expressed as relative light unit (RLU). A positive result from the HC2 test is defined as a net fluorescent activity that is greater than or equal to the fluorescent activity average of three manufacture positive controls. HC2 high-risk HPV DNA cut-off was 1.0 pg/mL, which is equivalent to 1,00,000 copies of HPV DNA.

VIA test and Colposcopy examination were done in the VIA and Colposcopy Center of BSMMU. Cytological and histopathological investigations were conducted at the Department of Pathology, BSMMU by conventional methods.

Statistical analysis:
Data obtained from the study were entered and analyzed by computer-based software SPSS Version 12. Test of significance was estimated by using Z test. Probability less than 0.05 were considered as significant.

Results

Cervical samples from a total of 68 women were tested for HPV DNA by the HC2 assay. The age of the study population ranged from 25-70 (mean 41.4±10.6) years. HPV DNA was detected from 13 (19.1%) samples. The prevalence of HPV infection increased with increasing age, with twice the prevalence among older than younger age groups. The maximum number of HPV DNA (26.7%) were detected in the >40 years age group. In the 25-40 years age group, HPV DNA was detected from 5 (13.2%) samples (Table-I). No statistically significant age difference was observed among the age groups (p=0.080).

HPV viral load ranged from 105 x 10^3 to 501 x 10^3 copies /ml among the study population. The highest viral load (501 x 10^3 copies /ml) was detected in one invasive carcinoma patient, while the lowest viral load (105 x 10^3 copies/ml) was detected in a case of chronic cervicitis. The mean viral load was 119.25 x 10^3±12.5 x 10^3 copies/ml (range: 110 x 10^3-137 x 10^3) in CIN I while this was 208.50 x 10^3±0.59 x 10^3 copies/ml (range: 139 x 10^3 - 305 x 10^3) for CIN II/ III. HPV DNA was detected from a patient with normal cytology with a viral load of 133 x 10^3 copies /ml (Table-II).

Table I: Rate of detection of HPV DNA among different age groups of study population.

Age (in years)	Total no. of cases (n = 68)	HPV DNA positive	p value
25-40	38	5 (13.2)	
>40	30	8 (26.7)	0.080
Total	68	13 (19.1)	

Note: i) z test was done to measure the level of significance.
ii) Figures within parenthesis indicate percentage.

Table II: HPV viral load in different types of cervical lesions.

Status of Cases	No. of cases (n=12	Viral load (copies/ml)	Mean Viral load Copies/ml.±SD	Range
Chronic Cervicitis	1	105 x 10³		
CINI	4	137 x 10³	119.25 x 10³	110 x 10³-
		119 x 10³	±12.5 x 10³	137 x 10³
		110 x 10³		
		111 x 10³		
	6	139 x 10³	208.50 x 10±	139 x 10³-
CINII/III		171 x 10³	0.59 x 10³	305 x 10³
		201 x 10³		
		305 x 10³		
		188 x 10³		
		247 x 10³		
Invasive carcinoma	1	501 x 10³		
Normal Cytology	1	133 x 10³		

Note:i) HC2 high-risk HPV DNA test cutoff 1 pg/ml is equivalent to 100000 HPV copies/ ml. RLU/ cutoff value ratios ≥1.0 was considered as "Positive".
ii) Viral load in HPV positive cases were considered only.
iii) No abnormal cytology was found in one patient with positive HPV DNA test.

Discussion

Human papilloma virus (HPV) is considered as the main cause of most cervical cancers and cervical intraepithelial neoplasia (CIN), and is thus an important public health challenge for the prevention of cervical carcinoma[16-17]. Traditional cytology using Papanicolaou (Pap) smear has played a major role in reducing mortality from cervical cancer. However, high rates of false negative results remains a major limitation of traditional cytological screening[18]. The HPV DNA testing has opened the door for an alternative surveillance mechanism to routine cytological screening. In this study, we detected HPV DNA from 13 (19.1%)) patients with various grades of cervical lesions. The overall and age-specific prevalence of HPV among women appears to vary by countries, region within countries and population subgroups. In Latin America, the frequency range was between 15% and 16% in Mexico, Costa Rica and Colombia[19-21]. Large studies have found 16.7 per cent of all screened women to be HPV DNA positive[22]. These geographical variations may be due to the prevalence of different subtypes of HPV and host related factors[23]. Moreover, demographic, cultural, socioeconomic variables, multiparity, long term contraceptive use, young age at first coitus, multiple sexual partners, low socioeconomic status, low education level, poor genital hygiene, cigarette smoking, genital tract infections etc, are probable co-factors that increase the risk of cervical cancer in women with HPV infection[24-25].

An important finding of our study was the age-related distribution of HPV lesions. While the prevalence of HPV was 13.2% among the 25-40 years age group, this increased two-fold to 26.7 % among the above 40 years age group. Almost similar results have been reported from Venezuela where 27% of HPV infection was detected in women between 45 to 54 years and 20% in women below 25 years[26]. Age-specific prevalence estimates show that HPV infection in women is predominantly acquired in adolescence, but peak prevalence in middle-aged women differ widely across geographical regions mainly due to differences in sexual behavior. However, HPV prevalence remains fairly constant across all age groups in high-prevalence countries of Asia and Africa[27]. Most studies observe that HPV infection usually reaches a peak of 20% among women between 20 to 24 years of age, with a subsequent decline to approximately 3% among women over 30 years[28-29]. Despite a decline in HPV prevalence above the age of 25 years, the risk for cervical cancer increases until women reach their fifties, probably due to risks associated with persistent HPV infection. Women over 30 years of age who are infected with high-risk HPV may be up to 116 times more likely to develop severe dysplasia than uninfected women[28]. Other factors regarding the progression of HPV infection to cervical cancer is related to a woman's immune status. Women with compromised immune system due to malnutrition, pregnancy, immunosuppressive chemotherapy, or co-infection with the human immunodeficiency virus (HIV) are at increased risk of progression[30-31].

In this study, a distinct upward trend of high-risk HPV DNA viral load was found to correlate with the histologic grade of the lesion, being highest for invasive carcinoma followed by CIN I, CIN II/II and lowest for chronic cervicitis . Here the highest viral load (501 x 10³ copies/ml) was detected in a patient with invasive carcinoma, while the lowest viral load (105 x 10³ copies/ml) was detected from a case of chronic cervicitis. The mean viral load in CIN I was 119.25 x 10³±12.5 x 10³ copies/ml (range: 110 x 10³ - 137 x 10³) and 208.50 x 10³ ± 0.59 x 10³ copies/ml (range: 139 x 10³ - 305 x 10³) in CIN II / III (Table-II). Sun et al. described Women who had a high viral load, according to the hc2 test, were found to be at significantly greater risk squamous intraepithelial lesion (SIL) and cervical carcinoma[13]. Various researchers have speculated that there may be a relationship between high-risk HPV DNA viral load with persistent infection and the subsequent development of high squamous intraepithelial lesion (HSIL) and invasive cervical carcinoma[32]. Some studies have

also shown that the amount of HPV DNA is a useful predictor of progression to cervical carcinoma, and concluded that the risk of cancer increased correspondingly[33]. We found the quantative information provided by the hybrid capture II assay to be quite accurate, reliable and reproducible. Thus, the quantification of viral load of high-risk HPV types may be a useful tool for patients with suspicious cervical lesions. An interesting case in our study was the detection of high–risk HPV DNA from a patient with no signs of any cervical lesion. The significance of the relatively high copy number of HPV DNA in the sample with normal cytological findings could not be addressed in this cross-sectional study. HPV DNA is presumed to be associated with latently infected cells, however, sampling errors may also be considered. Prospective studies of cytologically normal women using a quantitative analysis of HPV DNA may help to determine the natural history and biology of latent infections. However, there have also been conflicting reports about correlation between viral load and risk of SIL[34]. Thus, the quantitative aspects of HPV viral load testing merits further evaluation in order to establish its potential for cancer prevention in risk groups.

Sample size of this study is small due to its high cost and lack of awareness of HPV infection in suspected cervical carcinoma patients. However, further extensive studies with larger sample size should be carried out for use of this test for screening purpose. The main limitation of the HPV test is its high cost, which limits its use in routine cervical mass screening in resource limited settings. Nevertheless, it has been shown to be a useful tool when combined with cytology, diagnosing high-risk infections in apparently normal tissues, which may ultimately help reduce the risk of cervical cancer.

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