Original Paper

Correlation of HPV-DNA Test with Cytology and Histology for the Diagnosis of Cervical Cancer and Precancerous Lesions in a Tertiary Care Hospital in Bangladesh

Islam S[1], Uddin N[2], Akter N[3], Tabashum T[4], Sadakin MU[5], Jahan S[6], Khan KH[7]

Abstract

Introduction: Cervical cancer is the second most prevalent cancer among women worldwide. High-Risk Human Papilloma virus (hr-HPV) is an established cause of cervical cancer and precancerous lesion. Studies have shown the relationship between the HPV-DNA test with cervical precancerous and cancerous lesion in diagnosing cervical cancer. This study aimed to correlate HPV-DNA test with both cytology and histology.

Objective: To find out the correlation of HPV-DNA test with cytology and histology for the diagnosis of cervical precancerous and cancerous lesion.

Materials and Methods: Cervical smears and DNA samples were collected from the selected patients attending the colposcopy clinic of Bangabandhu Sheikh Mujib Medical University (BSMMU) from July 2011 to April 2013. Biopsy was done on colposcopically positive cases and histopathology reports were obtained. Thus 99 histologically proven patients of cervical cancer and precancerous lesion were selected. Pap smear was carried out on these 99 patients. HPV-DNA test (Hybrid Capture-2 assay) was carried out on the same samples and viral loads were estimated.

Results: Among the 99 cases, 28(28.28%) cases were positive with Hybrid Capture-2(HC-2) assay. Out of 60 cases of CIN-I (Cervical Intraepithelial Neoplasia-1), 7(11.7%) cases were positive with HC-2. Among others, 3(15.0%) cases of CIN-II were positive with HC-2. The viral load was very high in invasive SCC (squamous cell carcinoma) cases in contrast to other categories of histological and cytological diagnosis. A significant relationship was observed between HC-2 and histological diagnosis (P<0.005); and between HC-2 and Pap smear (P<0.005).

Conclusion: Introduction of HC-2 where possible along with Pap smear would be highly effective in primary screening and subsequent follow up of cervical precancerous and cancerous lesion.

Key-words: HPV, DNA, Cervical Cancer, Cancerous Lesion.

Introduction

Cervical cancer is the second most prevalent cancer among women worldwide[1]. High-Risk Human Papilloma virus (hr-HPV) is an established cause of cervical cancer and precancerous lesion[2]. The age-specific incidence rates of cervical cancer in Bangladesh are highest compared to Southern Asia and the World and the annual mortality rate is 11.6/100,000 women[3]. In Bangladesh, 50.19 million women are at risk of developing cervical cancer. Each year, 17,686 women are diagnosed with cervical cancer and 10,364 die from the disease[1]. A recent study in Bangladesh reported that cervical cancer accounted for 28.9% incidence and 17.9% mortality of all cancer cases in Bangladesh[4]. HPV-DNA can be detected by target amplification like PCR, signal amplification like HC-2 or probe amplification or ligase chain reaction[5]. The reported sensitivities of HC-2 varied in different settings. Some reported a high sensitivity of 97.9% (Arbyn et al)[6] while others[7,8] did not.

The primary diagnostic tools for HPV-related cervical lesion have been cytology and histology. Later, molecular methods to detect HPV DNA sequences in clinical specimens have been introduced[9]. Sankaranarayanan et al reported that the sensitivity and specificity of conventional cytology to be moderate (44–78%) and high (91–96%) respectively[10]. Tsai HT and co-workers reported a significant correlation between HPV-DNA test and Pap smear[11].

1. **Dr Shamina Islam**, MBBS, MD (Pathology), Assistant Professor of Pathology, East West Medical College, Dhaka 2. **Brig Gen Md Nizam Uddin**, MBBS, FCPS (Microbiology), Professor of Microbiology, Armed Forces Medical College, Dhaka 3. **Dr Nasima Akter**, MBBS, MD (Pathology), Assistant Professor of Pathology, East West Medical College, Dhaka 4. **Dr Tarana Tabashum**, MBBS, MD (Pathology), Assistant Professor of Pathology, East West Medical College, Dhaka 5. **Dr Maznun-Us-Sadakin**, MBBS, MPhil (Biochemistry), Lecturer of Pathology, Shaheed Tajuddin Ahmad Medical College, Gazipur. 6. **Dr Shahanaz Jahan**, MBBS, MD (Pathology), Assistant Professor of Pathology, Community Based Medical College, Mymensingh 7. **Professor Kamrul Hasan Khan**, MBBS, MPhil (Pathology), Department of Pathology, Bangabandhu Sheikh Mujib Medical University, Dhaka.

In Bangladesh, few studies have been published on the relationship between HPV-DNA test with cervical precancerous and cancerous lesion. This study aimed to correlate HPV-DNA test with both cytology and histology.

Materials and Methods

This descriptive cross-sectional study was conducted in the Department of Pathology in collaboration with the Department of Virology and Gynaecology & Obstetrics at Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka from July 2011 to April 2013. After collecting relevant history, 200 clinically suspected married patients of different age groups (18-60 years) were preliminarily selected. At first, cervical smears and DNA samples (cervical secretion collected by DNA sampler) were collected from all these 200 patients. DNA samples were stored at-200C. After that, VIA (vaginal inspection with acetic acid) was performed. Colposcopic biopsy was obtained from 120 VIA positive patients by the gynaecologist. When all biopsy reports were available, 99 cervical cancer and pre-cancer cases were finally segregated. Pap staining and HC-2 assays were performed with the samples of these 99 patients. Reporting of the Pap smears were done according to the Bethesda System and histopathological examination was done according to the standard protocol followed in the department of Pathology at BSMMU. HC-2 (Digene Corporation HPV Test-IVT) assay was carried out according to the manufacturer's protocol. All data were recorded in a predesigned data sheet. Statistical analysis of the result was obtained using Statistical Packages for Social Science (SPSS-21).

Results

The HC-2 was carried out on all 99 histologically positive cases. Among the 99 cases, 28(28.28%) cases were positive and the rest 71(71.72%) cases were negative by HC-2 assay (Table-I).

Table-II shows the correspondence of histological diagnosis and HC-2. Out of 60 cases of CIN-I (Cervical Intraepithelial Neoplasia-1), 7(11.7%) cases were positive with HC-2. Among others, 3(15.0%) cases of CIN-II were positive with HC-2. But for invasive squamous cell carcinoma (SCC) 18(94.7%) out of 19 cases were positive with HC-2.

Table-III reveals that only 2(4.0 %) out of 50 NILM cases were detected positive with HC-2. Among others, 5(21.8%) out of 23 cases of ASCUS and 17 out of 18 cases (94.4%) of invasive squamous cell carcinoma were positive with HC-2. Five cases of LSIL, 2 cases of HSIL were detected positive by HC-2 assay. One case of ASC-H was diagnosed negative by HC-2. The overall positivity of HC-2 was 28.28%.

The comparison of sensitivity and accuracy of Pap smear cytology and HC-2 are revealed in Table-IV. Sensitivities of Pap smear cytology and HC-2 were 49.5% and 28.3% respectively. The accuracy of smear cytology and HC-2 were 66.44% and 58.2% respectively. As the number of true negative samples is zero, specificity could not be estimated.

Table-I: Diagnosis of Cervical Lesions by HPV DNA Test (HC-2)

HPV DNA(HC-2) Test	Number of Patients	%
Positive	28	28.28
Negative	71	71.72
Total	99	100.0

Table-II: Comparison between HPV DNA test (HC-2) and histological diagnosis (n=99)

Histological Diagnosis	Total Cases	HPV DNA (HC-2) Test	
		Positive n(%)	Negative n(%)
CIN-I	60	7 (11.7)	53 (88.3)
CIN-II	20	3 (15.0)	17 (85.0)
Invasive SCC	19	18 (94.7)	1 (5.3)
Total	99	28 (28.28)	71 (71.72)
Significance	**Fisher's exact Test. P value < 0.005**		

HC-2: Hybrid Capture-2, CIN: Cervical intraepithelial neoplasia, SCC: Squamous cell carcinoma.

Table-III: Comparison of HPV-DNA test (HC-2) and cytological diagnosis (n=99)

Cytological Diagnosis	Number of Patients	HPV DNA (HC-2)Test	
		Positive n (%)	Negative n (%)
NILM	50	2 (4.0)	48 (96.0)
ASCUS	23	5(21.7)	18(78.3)
ASC-H	1	0 (0.0)	1 (100.0)
LSIL	5	2 (40.0)	3 (60.0)
HSIL	2	2 (100.0)	0 (0.0)
SCC	18	17 (94.4)	1 (5.6)
Total	99 (100.0)	28 (28.3)	71 (71.7)
Significance		**Fisher's Exact Test P value < 0.005**	

NILM:Negative for intraepithelial lesions and malignancy, ASCUS: Atypical squamous cell of undetermined significance, ASC-H:Atypical squamous cell-can't exclude HSIL, LSIL:Low grade squamous intraepithelial lesions, HSIL:High grade squamous intraepithelial lesions

Table-IV: Statistical analysis of Pap smear cytology and Hybrid Capture-2 (HC-2) assay

Statistical value	Pap smear cytology	HC-2 assay
Sensitivity	49.5%	28.3%
Accuracy	66.4%	58.2%

Discussion

The most widely used test for the detection of a group of 13 hr-HPV genotypes is the commercially available, FDA-approved HC-2 test Qiagen (formally known as Digene). HC-2 technology consists of a nucleic acid hybridization assay with signal amplification that utilizes microplate chemiluminescence for the qualitative detection of HPV. HC-2 has to be superior to Pap smear screening in reducing cervical cancer mortality[12].

Among the 99 histologically positive cases, 28(28.3%) cases were positive by HC-2 assay. Out of 60 cases, 7(11.7%) cases of CIN-I and 3(15.0%) out of 20 cases of CIN-II were positive with HC-2. But for invasive SCC 18 (94.7%) out of 19 cases were positive with HC-2. The overall sensitivity of HC-2 was 28.3% which correlates well with Saini et al[7] and Cremoux et al[8] who reported 36.4% and 26.89% sensitivities respectively. A significant relationship was observed between HC-2 and histological diagnosis (P<0.005).

While correlating HC-2 with cytological diagnosis, only 2 (4.0 %) out of 50 NILM, 5(21.8%) out of 23 cases of ASCUS and all 17 out of 18 cases (94.4%) of invasive squamous cell carcinoma were positive. Tozetti et al[13] reported the positivity of NILM, LSIL and HSIL to be 38.2%, 52.1%and 0.9% respectively. For SCC, Ashrafunnesa et al[2] and Cremoux et al[8] reported the positivity of 96.7% and 92% respectively which are almost similar to the present study. However, a significant relationship was observed between HC-2 and cytological diagnosis (P<0.005).

The comparative analysis of the sensitivity and accuracy of Pap smear cytology and HC-2 was carried out in this study and Pap smear showed better sensitivity than HC-2. The sensitivity and accuracy of Pap smear cytology are 49.5% and 66.4% respectively. While those of HC-2 assay were 28.3% and 58.2% respectively. In this study, the number of true negative samples is zero, because all 99 samples were obtained from histologically proven cases of cervical cancer and precancer. Due to this fact specificity of Pap smear and HC-2 could not be estimated.

Conclusion

In this study, although the sensitivity of HC-2 was not that high, significant correlations have been observed between HC-2 and histological findings; and HC-2 and Pap smear. Stringent DNA sample collection and storage is necessary for a better result. However HC-2 assay can be a useful adjunct with Pap smear during primary screening and follow up in resource-rich settings.

References

1. Yusuf N, Ali MA, Rahman ML et al. Screening of cervical cancer by VIA among women in Rajshahi Medical College Hospital. Asian Pacific Journal of Tropical Disease 2012; 2(1):70-2.

2. Ashrafunnessa, Khatun S, Haq F et al. Human Papillomavirus in Cervical Cancer in Bangladesh. Bangladesh J obstetGynaecol 2006; 21(2):51-7.

3. WHO/ICO Information Centre on HPV and Cervical Cancer (HPV Information Centre). Human Papillomavirus and Related Cancers in Bangladesh. Summary Report 2010 [Date accessed]. Available at www. who. int/ hpvcentre.

4. Khatun S, Akram Hussain SM, Chowdhury S et al. Safety and Immunogenicity Profile of Human Papillomavirus-16/18 AS04 Adjuvant Cervical Cancer Vaccine. A Randomized Controlled Trial in Healthy Adolescent Girls of Bangladesh. Jpn J ClinOncol 2012; 42(1):36-41.

5. Malloy C, Sherris J, Herdman C. HPV DNA Testing: Technical and Programmatic Issues for Cervical Cancer Prevention in Low-Resource Settings 2000.5-9. Available at http://www.path.org/publications/files/HPV-DNA-Testing-Issues.

6. Arbyn M, Sasieni P, Meijer CJLM et al. Clinical applications of HPV testing: A summary of meta-analyses. Vaccine 2006; 24:78–89.

7. Saini R, Shen TH, Santhanam J et al. Comparison of DR. HPV™ Chip Kit with hybrid capture II assay for the detection of human papillomavirus in clinical samples: a preliminary study. Tropical Biomedicine 2007; 24(1):17–22.

8. Cremoux PD, Coste J, Xavier SG et al. Efficiency of the Hybrid Capture 2 HPV DNA Test in Cervical Cancer Screening. Am J ClinPathol 2003; 120:492-9.

9. Burd EM. Clinical microbiology reviews 2003; 16:Pp.1-17. DOI:10.1128 / CMR.16.1.1-17.

10. Denny L, Michael QM and Sankaranarayanan R. Screening for cervical cancer in developing countries. Vaccine 2006; 24(3):71–7.

11. Tsai HT, Tsai YM, Yang SF et al. Comparison of Pap Smear Screening and Hybrid Capture II Strategies for Prediction of Cervical Intraepithelial Neoplasm. Epidemiology 2007; 18(5):S13.

12. Origoni M, Cristoforoni P, Costa Set al. HPV-DNA testing for cervical cancer precursors: from evidence to clinical practice. Ecancer 2012; 6:258. DOI: 10.3332/ecancer 2012.258.

13. Tozetti IA, Scapulatempo IDL, Levi JE et al. Determination of HPV DNA viral load by hybrid captures assay and its association with cytological findings. J Bras Patol Med Lab 2006; 42(6):449-53.