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Original article

Comparison of Hybrid Capture II (HCII) assay with conventional methods for diagnosis of Human Papilloma Virus (HPV) infection.

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Abstract:

Human papilloma virus (HPV) is the most prevalent virus involved in sexually transmitted diseases worldwide, and an important public health challenge for the prevention of cervical carcinoma. The present study compared the hybrid capture II assay with conventional methods for the diagnosis of HPV infection. A total of 68 women between 25-70 years of age were included in this study. Conventional methods used for diagnosis of the patients included Visible Inspection of Acetic acid (VIA), Histopathology and Pap smear tests. The hybrid Capture 2 assay was used to detect HPV DNA viral load in these patients. VIA test was performed on 66 patients. HPV DNA was detected in 8 (20.5%) of the VIA positive cases, 2 (9.5%) VIA negative, and 2 (33.3%) cases where VIA test were unsatisfactory. Pap smear test was done on 43 patients. Among them HPV DNA was detected in 13(19.1%) cases, of which 11 (37.9%) cases were Pap positive/abnormal and 2 (14.3%) were Pap negative. Out of 29 cases where histopathological examination was carried out, HPV DNA was detected in 1 (7.7%) chronic cervicitis case, 4 (66.7%) cases of CIN I, 7 (70.0%) cases of CIN II/III and invasive carcinoma. From this study it is suggested that in combination with conventional methods, hybrid Capture 2 assay is a useful tool to diagnose high-risk HPV infections when conventional tests shows apparently normal results.

Keywords: Human papillomavirus (HPV) , Hybrid Capture II, Papanicolaou (Pap) smears test, cervical carcinoma.

Introduction:

Worldwide, cervical carcinoma ranks second among the common cancers in women. [1] Human papilloma virus (HPV), a member of the papovaviridae family, is an oncogenic DNA virus which is associated with cervical cancer. This virus is predominantly sexually transmitted and is a high–risk factor for development of cervical carcinoma.[2-4]Persistent infection with certain genotypes of carcinogenic HPV is associated with nearly all cases (99.7%) of cervical cancers [5]Among the 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 and HPV types 31, 33, 35, 45, 52 and 58 are responsible for an additional 20% of cases. [6]

Inspection with acetic acid (VIA) and microscopic examination of papanicolaou (Pap) smears which detect abnormal cervical epithelium cells are the methods of choice for mass screening and enable early detection of lesions for effective treatment. Moreover, VIA is less effective for screening women in their fifties because of the tendency for the squamo-columner junction to recede in to cervical os, making observation of lesions difficult. [7]

The Pap smear screening is effective in preventing cervical cancer since the majority of cervical cancers are preceded by pre-cancerous lesions. However, it has some disadvantages; the most important ones are its limited sensitivity for detecting cancer precursors, and the subjective interpretation of results.

As a consequence, this test provides 20-30% false–negative results. [8] Moreover, Pap test needs frequent repetition, which raises the cost considerably and results in excessive interventions. [9]

Screening for cervical neoplasia using the Pap test alone is unreliable and additional methods should be used to improve the accuracy of routine diagnosis of cervical lesions. [10] Histopathology can be used to confirm most diagnosis by observing characteristic pathologic features of HPV infection. Molecular detection of HPV DNA is currently the gold standard for identification of HPV. HPV DNA test is being evaluated as a potential alternative or adjunctive to cervical cytology for the early detection of cervical cancer precursors and prevention of invasive cervical cancers.[11] In the present study, we compared hybrid capture II assay with conventional methods for the diagnosis of HPV infection.

Patients and methods:

The study was carried out among 68 women attending in the Gynecology Out-patients Department (OPD) of BSMMU Hospital from January to December 2008.The inclusion criteria of the study population were:

1. Sexually active women above 25 years of age with history of post-coital bleeding, per-vaginal spotting and /or spontaneous bleeding;
2. Patients with low-grade squamous intra-epithelial lesions (LSIL)
3. Clinically unhealthy looking cervix on per-vaginal examination.

VIA test were done at the VIA Center of Department of Obstetrics and Gynaecology, BSMMU. Cytological and histopathological investigations were conducted at the Department of Pathology, BSMMU. The HPV DNA test was done at the Department of Virology, BSMMU, Dhaka.

After taking informed written consent, cervical specimen was collected in a cervical sampler using aseptic technique. At the testing laboratory, specimens were stored at –20° C until the test was performed.

VIA involved swabbing the cervix with 3-5% acetic acid solution prior to visual examination. (Differences in precancerous cell structure and absorption rates make abnormal cells temporarily turn white when exposed to the solution.) The Pap smear test involved scraping cells from the cervix and then fixing on a glass slide. Slides were then sent to the cytology laboratory and evaluated by a trained BSMMU cytologist.

For histopathological examination specimens were obtained by punch biopsy after application of 3% acetic acid. The samples were collected in container 10 % formaline as fixative. Routine tissue processing with paraffin impregnation was done. For microscopic examination routine paraffin sections were stained with hematoxylin and eosin staining method.

HPV DNA was detected by 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. [12] The group of cancer causing viruses tested includes HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68.

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:

Out of the 68 women in this study, HPV DNA was detected from 13 (19.1%) and undetected in 55 (80.9%) cases.

Comparison between VIA and HPV DNA test was performed on 66 out of the total 68 cases. Of these, 39 (59.0%) were VIA positive, 21 (31.8%) were VIA negative while VIA was unsatisfactory in 6 (9.0%) cases. HPV DNA was detected in 8 (20.5%) VIA positive cases, 2 (9.5%) VIA negative cases and in 2 (33.3%) VIA unsatisfactory cases. **(Table-1).** No statistically significant difference (p=0.14) was observed among VIA positive and VIA negative cases.

Table-1: Relationship between status of VIA test and HPV infection.

VIA test	Total no. (n=60)	HPV DNA positive	P value
Positive	39	8(20.5)	0.14
Negative	21	2(9.5)	

Note: i) VIA test was unsatisfactory in 6 cases; HPV DNA test was positive in 2 (33.3%) of these cases. Statistical

analysis for unsatisfactory cases was not done due to small sample size. ii) Figures within parenthesis indicate percentage. Among the 43 study patients in whom pap smear test was done, 29 (67.4%) had abnormal paps result, while 14 (32.6%) were cytologically negative for intraepithelial lesions. Of the 29 abnormal paps cases, 3 (10.3%) were diagnosed as low grade squamous intraepithelial lesions, 5 (17.2%) cases had high grade squamous intraepithelial lesions, 1 (3.4%) case was diagnosed as squamous cell carcinoma, and 20 (68.9%) cases were diagnosed as atypical squamous cells of undetermined significance (ASCUS). HPV DNA was detected from 2 (14.3%) cases out of the 14 normal Pap cases and from 11 (37.9%) of the abnormal Pap cases. There was no statistically significant relationship between normal and abnormal pap results and positive HPV DNA cases (p=0.057). Out of 20 ASCUS cases, 4 (20%) were HPV DNA positive. HPV DNA was detected in 2 (66.7%) and 4 (80%) cases low-grade squamous intraepithelial lesions of high-grade squamous intraepithelial lesions. The single case of squamous cell carcinoma was HPV DNA positive. **(Table – 2)**

Table-2: Comparison of results of pap smear and HPV DNA test.

Pap smear results	Total no. (n=43)	HPV DNA positive	P value
Normal pap results	14	2 (14.3)	0.057
Abnormal pap results*	29	11(37.9)	
*Abnormal pap results	3	2 (66.7)	
a) Low grade squamous intraepithelial lesions (LSIL)/ CINI			
b) High grade squamous intraepithelial lesions(HSIL)/ CIN II/ III	5	4 (80.0)	
c) Squamous cell carcinoma (SCC)	1	1 (100.0)	
d) Atypical squamous cell undetermined significance (ASCUS)	20	4 (20.0)	

Note: Figures within parenthesis indicate percentage.

Comparison between histopathological examinations and HPV DNA test was carried out on 29 out of 68 study patients. Of these, 13 (44.8%) cases were diagnosed as chronic cervicitis, 6 (20.6%) cases as mild dysplasia (CIN I), 10 (34.5%) cases as moderate to severe dysplasia (CIN II/III) and invasive squamous cell carcinoma. HPV DNA was detected in 1 (7.7%) chronic cervicitis, 4 (66.7%) mild dysplasia (CIN I), and 7 (70.0%) moderate to severe dysplasia (CIN II/III) and invasive squamous cell carcinoma. (Table-3)

Table-3: Relation between histopathological findings and HPV DNA test of study population.

Histopathological status	Total no. (n=29)	HPV DNA positive
Chronic cervicitis	13	1(7.7)
Mild dysplasia / (CINI)	6	4 (66.7)
Moderate to severe dysplasia/ (CINII/III) and invasive squamous cell carcinom	10	7(70.0)
Not done	39	1 (2.6)

Note: Figures within parenthesis indicate percentage.

Discussion:

Human Papillomavirus infection is the main cause of cervical cancers and cervical intraepithelial neoplasias (CIN) worldwide.[13] Cervical cancer represents the second most common cancer in women globally, with 4,70,600 new cases and 233,400 deaths every year. [14] Well-organized screening programs have been effective in reducing the incidence of cervical cancer and preventing premature deaths. Screening is based on conventional methods eg, VIA, Pap smear and histopathology tests.

In our study, HPV DNA was detected in 8 (20.5%) VIA positive, 2 (9.5%) VIA negative and 2 (33.3%) VIA unsatisfactory cases. The detection of high-risk group HPV among 4 (14.8%) VIA negative and unsatisfactory cases helped in early diagnosis of pre-cancerous lesions in these patients. Some studies also reported that persistence of HPV infection is responsible for development of cervical pre-cancerous lesions and HPV DNA test can detect pre-cancerous lesions at its beginning. [7, 11,22]

Our study observed that HPV DNA was responsible for 66.7% low-grade squamous intraepithelial lesions (LSIL), 80.0% of high-grade squamous intraepithelial lesion (HSIL) cases, and

20% cases of atypical squamous cell undetermined significance (ASCUS). It was observed that HSIL was exclusively associated with high-risk HPV (Table- 2). Out of the 29 abnormal Pap results, HPV DNA was detected in 11 (37.9%) cases. There was no statistically significant relationship between normal and abnormal pap results and positive HPV DNA cases (p=0.057). Other investigators have reported HPV DNA in over 90.3% cases of LSIL, 85% - 90.4% cases of HSIL, 22.4% - 52.4% cases of ASCUS, and 84.6% SIL patients. [15-17]

Inconsistency between HPV DNA testing and cytology were observed in some cases in our study. This was probably due to errors in cytological diagnosis or true DNA positivity with no apparent abnormality rather than DNA testing errors. [18] Negative results in hybrid capture assay with alternations in Pap test results were also found. These inconsistent results could be due to low HPV genome copy numbers, infection by un-tested types, or due to sampling error and sampling inadequacy. [19]

In our study, HPV DNA was detected in 7.7% cases of chronic cervicitis, 66.7% cases of mild dysplasia (CIN I), 70.0% cases moderate to severe dysplasia (CINII/III), and invasive squamous cell carcinoma. Other studies revealed that high-risk types of HPV are responsible for 68.6% cases of CIN I and 93.3% cases of CINII/III. [11,15]

Several studies have shown that the performance of the HC2 assay is highly comparable and sometimes better than PCR for the detection of HPV DNA. [20, 21] HPV DNA by HC2 test may be related to less number of colposcopy which will also reduce transport cost, family interference and anxiety. [22]

HPV DNA test is a useful tool to diagnose high risk HPV infection in apparently normal cervical tissues. Women who are HPV DNA positive but do not have an abnormal Pap test or clinical disease should not be viewed as having false–positive test as evident from our study which detected HPV DNA in 2 (14.3%) cases of normal Pap test and 5 (13.2%) cases of negative colposcopy results. Indeed, these women are at great risk of developing abnormal Pap test and cervical neoplasia.

From the findings of this study , it can be concluded that when combined with conventional methods, HPV DNA test will greatly facilitate the early identification of women who are at risk of cervical cancer and thereby play a vital role in their management by close follow-up and repeat testing, which will eventually have a significant impact in reducing morbidity and mortality due to cervical cancers.

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