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"Screening of Cervical Cancer Acts as New Insights" - For Early Detection and Prevention by a Modern OncoE6 Protein Detection Assay

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Abstract

Cervical cancer is the most burning issue for women worldwide. It is the fourth leading type of cancer in women and increasing day by day. Persistent Infection with high-risk *Human papillomavirus* (HPV) is the main cause for the progression of cervical precancerous and cancerous lesion. The oncoproteins of HPV mainly E6 and E7 are responsible for the neoplastic alteration in epithelial tissues. The Objective of the study was screening of cervical precancerous and cancerous lesion of the patient attending at Mymensingh Medical College, Hospital. Following universal safety precautions, a total of 280 endocervical swabs were collected from VIA outdoor and Colposcopy Clinic of Obstetrics and Gynecology Department of MMCH between April 2016 to March 2017. In this study VIA, nested PCR and OncoE6 cervical test were done on 280 cases. A total of 24 (8.5%) cases were positive for HPV DNA by nested PCR and 21 (7.5%) cases were positive for OncoE6 protein by OncoE6 cervical test. On Histopathological diagnosis of 50 colposcopy positive cases 13 were diagnosed as cervical carcinoma among these 12 (92.30%) were positive for OncoE6 cervical test. Without early screening of cervical precancerous and cancerous lesion it is not possible to reduce cervical cancer in developing countries like Bangladesh. Screening of women by a novel oncoE6 protein test helps in early detection. By early detection it will be potential to give suitable treatment in appropriate time so that competent to save economy, time and many lives.

Introduction

Globally cervical cancer is silent cause of death for women and increasing gradually. Cervical cancer is recognized to progress from precancerous disease known as Cervical Intraepithelial Neoplasia (CIN) and it has been taken 5 to 15 years for advancement to invasive cancer. By broad epidemiologic and molecular biologic studies, the *Human papillomavirus* (HPV) infection is identified the most significant cause of cervical cancer [1].

HPV is a small, non-enveloped DNA virus with circular, double-stranded viral genome is approximately 8 kb to 12 kb in length that mostly infected skin or mucosal cells. The genome codes for 6 early proteins accountable for virus replication and 2 late proteins, L1 and L2, which are known as viral structural proteins. Till now above 120 types of HPV have been identified and categorized into high-risk and low-risk HPV group. The persistent infection of high-risk HPV is concomitant with progression of cervical cancer [2-4].

HPV is recognized to encourage cervical cancer by unrestrained G1-S transition. The E6 and E7 proteins of high-risk HPV deter the *p53* and pRb proteins which are cell cycle regulatory proteins monitoring G1-S transition [5]. More than 85% of cervical cancer deaths are in developing countries, where it accounts for 13% of all female cancers. Therefore, most of the burden of HPV-associated malignancy and indeed benign disease is in developing countries are due to absence of effective screening programs and poor access to medical services [6].

In Bangladesh, cervical cancer is the second most common cancer among females. The prevalence of HPV infection among Bangladeshi women has been reported to be 7.7% [7].

Human papillomavirus (HPV) is one of the most commonly acquired Sexually Transmitted Infections (STIs) and a significant source of morbidity and mortality.

Cervical screening programs using Pap smear testing have dramatically improved cervical cancer incidence and reduced deaths, but cervical cancer still remains a global health burden. The

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biomarker discovery for accurate detection and diagnosis of cervical carcinoma and its malignant precursors (collectively referred to as high-grade cervical disease) represents one of the current challenges in clinical medicine and cytopathology [8].

VIA is commonly used in Low and Middle-Income Countries (LMIC) achieving a moderate sensitivity with low specificity. In those areas with insufficient resources to manage large numbers of screen-positive women that would result from using a more sensitive but less specific HR-HPV DNA test, the OncoE6 test might be used for primary screening, thereby achieving a sensitivity similar or superior to VIA, which is already being widely used [9].

Limitations of DNA testing for HR-HPV include its complexity and cost and its inability to differentiate between low-risk and high-risk HPV infections. To address the limitation and increase access to screening, lower-cost test that targets HPV E6 oncoprotein has been developed. The prototype test is a lateral flow immunoassay that detects the E6 oncoprotein from HPV16 and 18 the 2 carcinogenic HPV genotypes that cause approximately 75% of cervical cancer [9]. This test is more specific for the detection of CIN3 or worse compared with HPV-DNA tests [10].

Methods

A Cross sectional observational study was carried out in the Department of Microbiology, Mymensingh Medical College, Mymensingh from January 2016 to December 2017. Informed written consent was taken from each patient before her entry into the study. Non-probability purposive type of sampling technique was used and sample size were (280) two hundred and eighty. Inclusion criteria was All the VIA negative and VIA positive married women attending at colposcopy clinic for cervical cancer screening with or without symptoms. Exclusion criteria for cases were patients who were pregnant, patients who were below 16 years of age, patients who were menstruating.

Laboratory Procedures

Collection of specimen

Cervical swab specimen was collected prior to application of acetic acid or iodine for colposcopy examination. Excess mucous was removed from the cervical os and surrounding ectocervix by using a cotton or dacron swab. For OncoE6 cervical test samples were collected from the endocervical opening and the ectocervix by inserting the swab just inside the cervical os so that the tip reached a depth of approximately 0.5 cm and by rotating the swab 3 turns in a counterclockwise fashion. Then the surface of the ectocervix was gently wiped. The specimen was stored in the supplied tube without buffer until tested. If the specimen was not used immediately, it was refrigerated at -20°C. Specimen was thawed for approximately 10 min at ambient temperature before being tested.

Procedure of OncoE6 cervical test

A cervical specimen collected using a polyester swab was stored in a tube without buffer until tested. The specimen was prepped sequentially by treating the swab with a lysis solution (930 µl) for 15 min, a conditioning solution (87 µl) for 15 min, and then clarifying the specimen solution using a table-top microcentrifuge (10 min at >10,000 rpm) to separate sample lysate from cellular debris. A 0.2 mL aliquot of the clarified specimen solution was then transferred into a Detector Reagent C vial with lyophilized detector monoclonal antibody alkaline-phosphatase conjugate. The test strip with immobilized capture monoclonal antibodies was inserted into Detector Reagent C vial containing specimen-conjugate mixture and the solution was permitted to migrate up the strip by capillary action. After 55 min, the test was washed by wash solution (200 µl) for 12 min and then immersed into the developing solution (650 µl) containing the alkaline-phosphatase substrate (Nitroblue Tetrazolium). After 15 min to 25 min (depending on the ambient temperature), the test unit was removed from the developing solution vials and placed on a reading guide, allowing for visual inspection. Appearance of one or more test lines indicates E6 oncoprotein of the corresponding HPV type present in the initial cervical swab specimen. Results must be read within 3 min.

Discussion

Out of 120 VIA positive cases, 18 (15%) were positive by OncoE6 cervical test (Table 1). In China Zao et al. [9] in his study had detected OncoE6 protein in 50.5% of VIA positive cases by OncoE6 cervical test. Nahar et al. [11] reported that among 47 VIA positive cases 21 (44.68%) were positive by OncoE6 cervical test.

In the current study among 160 VIA negative patients, 3 (1.86%) were positive by OncoE6 cervical test (Table 2). This result could not be compared with other studies. It was the first study that could detect OncoE6 protein from VIA negative cases. It indicates that OncoE6 cervical test was much better test than VIA for screening of cervical lesions.

The present study also revealed among 21 OncoE6 positive cases 4% (01/22) for CIN I, 37.5% (8/21) for CIN II, 71.42% (7/21) for CIN III and 92.30% (12/13) for cervical carcinoma were positive for OncoE6 protein (Table 3). Nahar et al. [11] found 01/11 (9.09%) CIN I case and 20/22 (90.90%) of cervical carcinoma were positive by OncoE6 cervical test. Zhao et al. [9] showed 8.5% and 84.6% were OncoE6 positive in CIN I and cervical carcinoma respectively which were similar with the present study. According to the study percentages of E6 positive increased steadily with increasing severity

Table 1: Detection of OncoE6 protein among VIA (n=120) positive cases by OncoE6 cervical test.

OncoE6 cervical test	Number of cases	Percentage (%)
Positive	18	15%
Negative	102	85%
Total	120	100%

Table 2: Detection of OncoE6 protein among VIA negative cases by OncoE6 cervical test (n=160).

OncoE6 cervical test	Number of cases	Percentage (%)
Positive	3	2%
Negative	117	98%
Total	120	100%

Table 3: Comparison between OncoE6 cervical test (n=21) and histopathological diagnosis (n=50).

Histological diagnosis	Number of cases	OncoE6 positive cases (%)
CIN-I	22	01 (04%)
CIN-II	8	03 (37.5%)
CIN-III	7	05 (71.42%)
Cervical carcinoma	13	12 (92.30%)
Total	50	21

Table 4: Detection of OncoE6 protein among total population by PCR (n=280).

OncoE6 cervical test	Number of cases	Percentage (%)
Positive	24	857%
Negative	256	91%
Total	280	100%

Table 5: Sensitivity and Specificity of OncoE6 cervical test considering PCR as gold standard.

Test result	OncoE6 cervical test		Total	Sensitivity	Specificity
	Positive	Negative			
PCR positive	21	3	24	87.50%	100%
PCR negative	0	256	256		
Total	21	259	280		

of lesions.

In the current study, PCR and OncoE6 cervical test were done on all the 280 cases as screening test (Table 4 and 5). The sensitivity and specificity of OncoE6 cervical test considering the PCR as gold standard were 87.5% and 100% respectively. According to Nahar et al. [11] the sensitivity and specificity of OncoE6 cervical test were 75% and 100% respectively which was similar with present study. The sensitivity was less due to OncoE6 cervical test only detected HPV-16 and HPV-18 type but unable to detect other high-risk HPV types which responsible for cervical cancer. In addition, the elevated expression of E6 is required for epithelial cell transformation to cause cancer. So, detection the OncoE6 proteins represents an attractive, disease specific viral biomarker.

Conclusion

It may be concluded that the OncoE6 test may be used for primary screening, thereby achieving a sensitivity similar or superior to VIA, which is already being widely used. However, because of its much higher specificity compared with VIA, screening with HPV E6 reduce either the number of referrals to colposcopy when diagnostic verification is required, thereby saving on scarce clinical and financial resources or overtreatment in the context of a screen-and-treat program. The use of this tool will accelerate the reduction of the cervical cancer burden while we wait for prophylactic HPV vaccines to reduce the population risk in the future.

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