Correlation between Sociodemographic Variables and OncoE6 Cervical Swab Test Positive Cases

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Abstract

A cross-sectional, observational study was conducted to determine the relationship between sociodemographic variables and the development of cervical cancer through detection of detection of HPV-E6 oncoprotein. A total of 47 endocervical swabs were collected from colposcopy clinic under the Department of Obstetrics & Gynaecology, Mymensingh Medical College, Mymensingh, Bangladesh, from January to December of 2015. Laboratory work was done in the Department of Microbiology of the same institution. E6 strip test is an immunochromatographic test based on detection of HPV-E6 oncoprotein in cervical swab samples. The swab specimen was treated with lysis solution and conditioning solution. Then the specimen solution was clarified by centrifugation. After that the sample solution was transferred into the detector mAb vial, wash solution vial, and finally, into developing solution vial. The test unit was then placed on a reading guide. Positive result was indicated by the appearance of purple colored test line. Out of 47 specimens, 21 (44.68%) were OncoE6 positive. Among those 21 positive cases, 19 (90.48%) were HPV-16 and 2 were (9.52%) HPV-18. Out of 11 histologically diagnosed CIN I cases, 1 (9.09%) case came out as positive. However, there were no positive cases by oncoE6 cervical swab test in CIN II and CINIII categories. Among histopathologically confirmed 22 cervical carcinoma cases, 20 (90.90%) came out as positive through this test. The results were compared with sociodemographic variables. OncoE6 is more common among ≥50 age group, and who got married before 18 years, and in multiparous women (P<0.05). Cervical cancer prevention services should be strengthen including health education, OncoE6 screening, and Anti-HPV vaccination.

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Introduction

Cervical cancer ranks as the fourth most frequent cancer among women which contributes to about 10% of all female cancers globally.[1] Current estimates indicate that every year 527,624 women are diagnosed with cervical cancer and 265,653 die from the disease. It is the second most common cause of cancer related deaths in women aged 15 to 44 years in the world.[1,2] Harald zur Hausen and his collaborators identified the connections of HPV 16 and HPV 18 infections and cervical cancer. They demonstrated that parts of the HPV DNA were integrated into the host genome in cervical cancer cell lines including the HPV-16 and HPV-18 viral E6 and E7 genes which were preferentially retained and expressed in the tumours.[3-5]

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Human papilloma viruses (HPV) belong to the Papillomaviridae family. The HPVs are relatively small, DNA viruses without an envelope; the diameter is 55 nm. They have icosahedral symmetry, 72 capsomers and a DNA which contains 6800-8400 base pairs. The virus genome codes 8-l0 proteins in which there are structured (L1 and L2) and non-structured (E1; E2; E4; E5; E6 and E7) proteins.[6] E6 and E7 are the most important oncogenic proteins. Inactivation of p53 and Rb (Retinoblastoma) tumor suppressor gene by E6 and E7 respectively results in uncontrolled proliferation of cells which play an important role in the development of cervical cancer cells.[7]

Cervical cancer is a multi-etiology disease and HPV infection alone is not a sufficient cause of cervical cancer.[8] More than 90% of cases spontaneously regress and in about 10% eventually progress to high grade lesions and even less frequently evolve to invasive.[9] HPV is predominantly sexually transmitted and is a high-risk factor for development of cervical carcinoma. The longtime frame between initial infection and evident disease indicates that other exogenous or endogenous cofactors, such as sexual reproductive factors, sexually transmitted diseases, nutritional deficiencies and fruits and genetic susceptibility, acting in conjunction with HPV may be necessary for the disease progression.[8]

The cofactors known to increase the incidence of cervical cancer are early marriage and sexual practice, delivery of the first baby before the age of 20, too many or too frequent childbirths, multiple sexual partners, poor practice of personal hygiene, low socioeconomic status, HIV positivity, long term use of oral contraceptive pills, tobacco smoking, etc.[10,11] Besides, co-infection with Chlamydia trachomatis and Herpes simplex virus type-2, immunosuppression and certain dietary deficiencies are other probable cofactors responsible for the process.[1,10,11] Therefore, the sociodemographic risk quantification is warranted to acquire a better picture of the determinants of cervical carcinoma in low resource settings like our country. With the above-mentioned background, the present study was carried out to determine the relationship between sociodemographic characteristics and OncoE6 cervical swab test positive cases.

Methods

This cross-sectional, observational study was carried out between January and December of 2015 in Department of Microbiology, Mymensingh Medical College, Mymensingh, Bangladesh. All VIA positive married women attending the colposcopy clinic under Department of Obstetrics & Gynaecology, Mymensingh Medical College, were selected as subjects. Informed written consent was taken from each study participant. Nonprobability purposive type of sampling technique was used. Sample size was forty-seven. Cervical tissue was taken by endocervical swab. Patients who were pregnant, or below 20 years of age, or menstruating were excluded from the study. A semi-structured questionnaires was used to collect participants' demographic information. After that, 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. Then swab samples were taken from the endocervical opening and the ectocervix by inserting the swab just inside the cervical os,

as 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 minutes at ambient temperature before being tested. The swab specimen was treated in a two-step process, first with 930µL of lysis solution and next with 87µL of conditioning solution, both with 15-minute incubation under gentle agitation. Then the specimen solution was clarified from insoluble components by centrifugation in a table-top microcentrifuge for 10 minutes at >10,000 rpm. After that a 200-µL aliquot of the sample solution was transferred into a vial with lyophilized detector mAb. The test unit was then inserted into the detector mAb vials, and the specimen solutions ran up the test strips by capillary action. After 55 minutes, the test unit was transferred into vials with wash solution, and after a 12-minute washing the test unit was immersed into another set of vials containing developing solution. After 15–25 minutes (depending on the ambient temperature), the test unit was removed from the developing solution vials and placed onto a reading guide, allowing for visual inspection. Appearance of one or more test lines indicated E6 oncoprotein of the corresponding HPV type present.

Laboratory results were also added to this data sheet. Data were systematically recorded, scrutinized, and compiled. Continuous variables were compared using unpaired student's t-test, while categorical variables were compared using Chi-square test. Level of significance was defined as P value <0.05 at 95% confidence interval.

Statistical analysis was done using Statistical Package for the Social Sciences (SPSS) version 20.0 for windows. This study was approved by the Ethical Review Committee of Mymensingh Medical College, Mymensingh, Bangladesh.

Results

OncoE6 cervical swab test done on total 47 VIA positive patients attending the colposcopy clinic. Among them, 21 (44.68%) patients were positive HPV-E6 oncoprotein (Table-I). Among them, HPV-16 and HPV-18 comprised 90.48% and 9.52% cases respectively (Table-II). Table-III represents the comparison of histological diagnosis with oncoE6 cervical swab test. Out of 11 histologically diagnosed CIN I cases, 1 (9.09%) case came out as positive. However, there were no positive cases by oncoE6 cervical swab test in CIN II and CINIII categories. Out of 22 histologically positive cervical carcinoma, 20 (90.90%) cases were positive. Most of the positive cases of study population were in the age group ≥50 years (42.86 %) followed by age group 30-39 years and 40-49 years (28.57 % each). No positive case was found in <30 years age group. The difference was statistically significant (P<0.05) (Table-IV). Among the positive cases, 20 (95.24%) got married at <18 years of age, while only one case was at >18 years; the difference was statistically significant (P<0.05) (Table-V). 17 women were positive having parity of >3, while only 4 women were positive having parity of ≤3. The difference was also statistically significant (P<0.05) (Table-VI).

Table-I: Detection of OncoE6 gene among VIA positive cases by cervical swab test (n=47)

Onco E6	Number of cases	Percentage
Positive	21	44.68
Negative	26	55.32
Total	47	100

Table-II: Distribution of HPV-16 and HPV-18 among OncoE6 positive cases in cervical swab test (n=21)

HPV type	Number of cases	Percentage
HPV-16	19	90.48
HPV-18	02	09.52
Total	21	100

Table-III: Comparison between OncoE6 cervical test and histological diagnosis (n=36)

Histological diagnosis	Number of cases	OncoE6 positive cases
CIN-I	11	1 (9.09%)
CIN-II	02	0 (0%)
CIN-III	01	0(0%)
Cervical carcinoma	22	20 (90.91%)
Total	36	21(100%)

Table-IV: Age distribution of OncoE6 positive cases

Age group	OncoE6		P value
	Positive	Negative	
<30 years	0	4	<0.05[S]
30-39 years	6	12	
40-49 years	6	5	
≥50 years	9	5	
Total	21	26	

P value reached from unpaired Student's t-test; S=significant.

Table-V: Distribution of OncoE6 positive cases according to age at marriage

Age at marriage	OncoE6		P value
	Positive	Negative	
<18 years	20	21	<0.05[S]
≥18 years	1	5	
Total	21	26	

P value reached from Chi-square test; S=significant.

Table VI: Distribution of OncoE6 cases according to parity

Parity	OncoE6		P value
	Positive	Negative	
≤3	4	18	<0.05[S]
>3	17	8	
Total	21	26	

P value reached from Chi-square test; S=significant.

Discussion

Cervical cancer is a major health problem globally; 85% of cases occurring in developing countries where cervical cancer is a leading cause of cancer death in women.[12] Persistent human papillomavirus infection is the most important factor in the development of cervical cancer.[10] Most HPV infections regress rapidly without causing clinically significant disease. A multitude of risk factors aggravate the disease.[8]

In our study, 47 VIA positive women were selected for OncoE6 cervical test. 21 (44.68%) out of 47 cases were positive by OncoE6 cervical test. OncoE6 detection was done on randomly selected suspected cases. Among 21 positive cases, 19 were HPV 16 and 2 were HPV 18. According to Yim & Park, high-risk HPV DNA is found in almost all cervical cancers (>99.7%), with HPV16 being the most prevalent type in both low-grade disease and cervical neoplasia which corresponds the present study by being more positive in HPV16.[13]

The result of histopathological examinations of 36 colposcopy positive cases were revealed that, 11 (30.56%) cases were CIN I, 02 (5.56%) cases were CIN II, 01(2.78%) case was CIN III and 22 (61.11%) patients were diagnosed as cervical carcinoma. Ansari reported that on histopathological examinations of 160 cases, 56 (35.00%) cases were chronic cervicitis,

51 (31.9%) cases were CIN I, 14 (8.8%) cases were CIN II and 36 (22.5%) cases were invasive squamous cell carcinoma.[14]

Our study also revealed that 9.09% CIN I, and 90.90% of cervical carcinoma were positive by OncoE6 cervical test. Zhao et al. showed 8.5% and 84.6% were OncoE6 positive in CIN I and cervical carcinoma respectively, which has similarity with the present study.[15] According to the study results the percentages of E6 positive increased steadily with increasing severity of diagnosis.

Another important finding of our study was the age-related distribution of HPV lesions. In the present study, most of the (42.86%) OncoE6 positive cases of study population were in age group ≥50 years, while 28.57% was found in 30-39 years and 40-49 years the age groups. According to Zhao et al., the highest percent positive was in women aged 50 years and older, which is similar to our finding.[15]

The parity distribution of the patients under the present study revealed that most (80.95%) of the Onco E6 positive patients fall under the category of grand multipara (>3) and 9.52% had parity of ≤3. Gudlevičienė *et al.* suggested that multygravidity is associated with the increased risk of carcinoma of cervix.[16] We found an association between multiparity and E6 oncoprotein, too.

In this study we compared all sociodemographic factors with the OncoE6 positive cases. Although high-risk HPV infection is more common among younger women, cervical cancer is primarily a disease of older, married (or previously married) women.[17] These differences reflect the time interval between infection and development of cancer, as well as the many other variables that influence the pathogenicity. The changing socio-economic profile, initiation of OncoE6 screening strategy and HPV vaccination may result in lowering the cervical cancer incidence and mortality.

Conclusion

Based on the findings of the present study, it may be concluded that OncoE6 is more common among ≥50 age group, and who got married before 18 years, and in multiparous women. The risk factors for HPV infection are those that the risk factors for OncoE6 positive cervical cancer. Cervical cancer prevention efforts in the vaccination era must ensure that all women with the risk factors have access to preventive services including education, OncoE6 screening, and HPV vaccines. Otherwise, existing disparities in cervical cancer could worsen.

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