

Value of PAP's Smear with Regard to DNA Testing for Identification of Cervical Precancerous Lesions in VIA Positive Cases

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Abstract

Background: Carcinoma cervix is one of the cause of female death in developing country like Bangladesh. Prevention can be done by several screening procedure like VIA, Pap's smear, HPV DNA testing, Colposcopy and colposcopy directed biopsy. We can markedly reduce the mortality and morbidity by these procedures and can be detected carcinoma in precancerous and very early carcinoma stage. This study was conducted to compare HPV DNA testing and pap's smear for identification of cervical precancerous lesions in VIA positive cases.

Materials and methods: It was an analytical type of cross sectional study. Data were obtained in outpatient Department of Gynecology and Obstetrics, Chattogram Medical College Hospital, Chattogram from July 2014 to December 2014. Total 90 subjects were included in the study consecutively after considering inclusion and exclusion criteria and taking written informed consent. Data analysis was done by SPSS (Version 17).

Results: Histopathology findings of biopsy materials of colposcopy positive cases where 8(28.6%) were chronic cervicitis, 10(35.7%) were CIN I, 7(25.0%) were CIN II, 3(10.7%) were CIN III. Total 20(71.4%) cases were found positive or precancerous. Regarding validity analysis of different tests that were performed for cervical precancerous conditions taking histopathology results as a gold standard, sensitivity of Pap's smear was found lower (75.0%) than the HPV DNA whereas specificity of Pap's Smear was higher (87.5%) than the specificity of HPV DNA (75.0%).

Conclusion: HPV DNA testing was both more sensitive and specific near to Pap cytology. So the use of a less invasive and more user friendly primary screening strategy like HPV-DNA testing may be required to achieve the coverage necessary for effective reduction in cervical cancer mortality.

Key words: Cervical Precancerous Lesion; DNA Testing; PAP's smear.

INTRODUCTION

Cancer of the cervix is a global health problem. 4,70,600 new cases occur worldwide each year, the vast majority of which are in developing countries¹. According to WHO current estimates indicates that every year 17,686 women are diagnosed with cervical cancer and 10,364 die from the disease in Bangladesh^{2,3}. All over the world cervical carcinoma is the second most common cancer in female after breast cancer. During the last 40 years mortality due to this cancer has been reduced significantly in developed countries and that is because of different screening tests such as Pap's smear and HPV DNA detection⁴. The HPV testing was the most objective and reproducible of all cervical screening tests and was less demanding in terms of training and quality assurance⁵. During the last decade, the role of Human Papilloma Virus (HPV) in development of cervical cancer and cervical precancerous lesions has been confirmed and a great number of articles concerning HPV detection

in cervical cancer screening has been published⁶. Despite the reduction, this disease is still one of the most important causes of mortality in women especially in developing countries^{1,4}. The prevention and control of cancer in developing countries deserve urgent attention. In limited resource setting WHO has recommended once in a life time screening for early detection of cancer in all women between 35 and 40 years of age. Pap's smear is the most commonly used screening method for early detection of cervical cancer, but it has low sensitivity (<50%) and a delay in providing result¹.

So it is important to use an adjunctive screening test like HPV DNA testing which has high sensitivity about 94.6% and can provide immediate result^{5,6,7}. Over the last decade, efforts to reduce the global cervical cancer burden through screening have focused on development and evaluation of alternative screening assays to the Pap's smear. Two such assays have been widely promoted: visual inspection of the cervix following acetic acid application (VIA) and molecular tests for the presence of high risk Human Papilloma Virus (HR-HPV) infection⁸.

Infection with sexually transmitted HPV types is more common in younger age groups, particularly among women in their late teens and twenties. Women who become sexually active at a young age, who have multiple sexual partners, and whose sexual partners have other partners are at increased risk of genital HPV infection. Most HPV infections are transient, or temporary, but sometimes an infection can remain detectable for many years. Seventy-five percent of sexually active people will be infected with the Human Papilloma Virus. (HPV) at some point in their lives. The virus is usually cleared by the immune system without treatment in less than two years in healthy people. Although both men & women can become infected, women are at greater risk for development of Human Papilloma Virus related cancer. Therefore, HPV testing is largely targeted at women. Infection by the Human Papilloma virus has no symptoms, and most people do not know that they have it. There is currently no treatment for HPV infection⁹.

The Human Papilloma Virus (HPV) DNA test identifies women who have an HPV infection. There are more than 30 types Human Papilloma Virus (HPV) that infect the anogenital (vulva, cervix, anus and penis) area. Based on the risk of causing cervical cancer, they are grouped into low-risk and high-risk categories. Low-risk HPV types cause genital warts and mild cervical cell changes, which are detected by a Pap's test. These changes are usually temporary. Infection by high-risk Human Papilloma Virus (HPV) types, especially when the infection persists for many years, can cause cervical cells to become precancerous and possibly cancerous.

It is appropriate that women 30 years of age or older have both the high-risk Human Papilloma Virus DNA test and a Pap's test at time of routine screening, as using both tests for women in this age group even further increases the chances for identifying precancerous changes. For women 30 years of age and older, if

both the high-risk Human Papilloma Virus DNA test and the Pap's test are negative, it is highly unlikely that a woman has precancerous changes or cancer. Because cervical cancer develops so slowly, when both tests are negative, routine cervical testing does not need to be repeated for three years. If the Pap's test is negative and the HR-HPV test is positive, both test should be repeated in 6-12 months. If both tests are positive additional testing is necessary⁹. Invasive cervical cancers are usually preceded by a long phase of preinvasive disease, characterized microscopically as a spectrum of precursor's lesions processing from cellular atypia to various grades of Cervical Intraepithelial Neoplasia (CIN) before progression to invasive carcinoma¹⁰.

Here all women with positive HPV DNA testing and abnormal cervical cytology should have colposcopic examination. The colposcopy provides a well-lighted magnified stereoscopic view of the cervix and its utilization in the evaluation of the cervix. Cervical intra epithelial neoplasia begins in the transformation zone, that area of the cervix in which native columnar epithelium is replaced by squamous epithelium. The colposcopic evaluation of the transformation zone and of the endocervical canal is a useful procedure in the evaluation of a patient with an abnormal cervical smear¹¹. Colposcopy is not recommended as a primary screening technique. It is too time consuming and expensive for use on a large scale.

The sensitivity of both tests used together was 100% and the specificity was 92.5%⁴. So now the American Cancer Society and the American College of Obstetrician and Gynecologists outline a screening strategy whereby women can have both a Pap's smear and a HPV test at the same time if the test negative for both, they would be asked to return for screening only at three years interval instead of yearly interval¹².

The necessity to develop optional diagnostic tools for cervical cancer screening, particularly in low resource settings, is widely recognized. Such potential screening tools include VIA, VILI, HPV testing, cervicography and possibly, screening colposcopy. There is no argument that organized cytological screening is the only cost-effective means of cervical cancer control, and should be used as the gold standard to which the other screening technology should be compared¹².

However, carcinoma cervix is one of the cause of female death in developing country like Bangladesh, especially women in this south east region are more conservative and ignorant. They are illiterate and hesitate to approach to medical persons for their health problems. So carcinoma cervix remains undiagnosed here until they are in advanced stage. Cervical cancer is a preventable disease. Prevention can be done by several screening procedure like VIA, Pap's smear, HPV-DNA testing, Colposcopy and colposcopy directed biopsy. We can markedly reduce the mortality and morbidity by these procedures and can be detected carcinoma in precancerous and very early carcinoma stage. Cervix is a surface organ, easily accessible, approachable and has a long pre-malignant phase, where treatment is also available. So long term follow up of a

woman by screening procedures can reduce cervical cancer in low setting resources. In our country VIA can be done in remote area by less skilled persons. So after VIA test positive cases should be referred to Tertiary Hospital for further evaluation by HPV-DNA detection and by Pap's smear. Following above screening procedures, the positive cases are subjective to do colposcopy directed biopsy from abnormal area. Colposcopy further helps in executing a targeted biopsy which can be useful in diagnosis of precancerous lesions and carcinoma cervix very early stages. In our knowledge we have got few study regarding value of Pap's smear with regard to DNA testing for identification of cervical precancerous lesion in VIA positive cases. Therefore this study tried to evaluate HPV DNA testing as a screening test among VIA positive cases.

MATERIALS AND METHODS

It was an analytical type of cross sectional study. Data were obtained in outpatient Department of Gynecology and Obstetrics, Chittagong Medical College Hospital, Chattogram from July 2014 to December 2014. Total 90 subjects were included in the study consecutively after considering inclusion and exclusion criteria and taking written informed consent. Data analysis was done by SPSS (Version 17).

Inclusion criteria

All VIA positive cases attending in the outpatient Department with the following criteria -

- i) Age between 30 to 65 years
- ii) Post coital bleeding
- iii) Post menopausal bleeding
- iv) Persistent vaginal discharge.

Exclusion criteria

- i) Known case of CIN
- ii) Subjects who will be menstruating
- iii) Pregnancy
- iv) Presence of a frank growth on cervix
- v) Subjects who had hysterectomy
- vi) Patients unwilling to give informed consent to take part in the study.

RESULTS

Table I showing mean age of the women were 39.3 years, mean duration of marital life is 21 years, age at first intercourse was 18.18 years. Obstetric variables of the study women where parity of most of them were 2-4 and 53.3% were on contraceptive pill.

Table II showing results of Pap's smear where NILM were found among 57.8%, inflammatory lesions were found in 22.2%, HSIL and LSIL were found in 15.6% and 4.4% respectively. Total 20.0% positive cases of Pap's smear were found in the study. Table III showing results of HPV-DNA analysis where 24(26.7%) cases were found positive and 66(73.3%) were found negative and Colposcopic findings where

aceto-white positive cases were 28(31.1%) and 62(68.9%) cases were negative.

Histopathology findings of biopsy materials of colposcopy positive cases where 8(28.6%) were chronic cervicitis, 10(35.7%) were CIN I, 7(25.0%) were CIN II, 3(10.7%) were CIN III. Total 20(71.4%) cases were found positive or precancerous (Table IV).

Table V showing association of positive results of HPV DNA and Pap's smear results. Significant association were found between them ($p < 0.05$). Regarding validity analysis of different tests that were performed for cervical precancerous conditions taking histopathology results as a gold standard, sensitivity of Pap's smear was found lower (75.0%) than the HPV-DNA (90.0%) whereas specificity of Pap's smear was higher (87.5%) than the specificity of HPV DNA (75.0%) (Table VI).

Table I : Condition of age and its relation with marriage among the Respondents (n = 90)

	n	Mean	SD	Median	Range
Age (Years)	90	39.93	6.96	40.00	30 – 60
Duration of Married Life (Years)	90	21.80	7.98	22.00	6 – 43
Age at First Intercourse (Years)	90	18.18	3.15	17.00	14 – 26
Marital Age (Years)	90	18.18	3.15	17.00	14 – 26

Table II : Results of Pap's smear study and interpretations among the Respondents (n = 90)

	Pap's Smear Findings	Frequency	Percentage (%)
Diagnosis	NILM	52	57.8
	Inflammatory	20	22.2
	HSIL	14	15.6
	LSIL	04	4.4
Interpretation	Positive	18	20.0
	Negative	72	80.0
Total		90	100.0

Table III : Results of HPV DNA and its interpretations among the Respondents (n = 90)

	HPV – DNA Findings	Frequency	Percentage (%)
Interpretation	Positive	24	26.7
	Negative	66	73.3
Interpretation	Colposcopic Findings		
	Positive / Aceto-white	28	31.1
	Negative / Normal	62	68.9

Table IV : Results of Histopathology findings of Biopsy materials among the colposcopy positive subjects (n = 28)

	Biopsy Findings	Frequency	Percentage (%)
Diagnoses	Chronic Cervicitis	08	28.6
	CIN I	10	35.7
	CIN II	07	25.0
	CIN III	03	10.7
Interpretation	Positive / Pre-cancerous	20	71.4
	Negative / Normal	08	28.6
Total		28	100.0

Table V : Association between Biopsy with Paps smear & HPV-DNA (n = 28)

		Biopsy Findings			p value
		Positive	Negative	Total	
Pap's Smear	Positive	15	01	16	0.001
	Negative	05	07	12	
HPV DNA	Positive	18	02	20	0.001
	Negative	02	06	08	
	Total	20	08	28	

Table VI : Evaluation of Pap's smear & HPV DNA detection as screening tests in respect to Biopsy as diagnostic test (n = 28)

Validity	Pap's Smear	HPV DNA Detection
Sensitivity	75.0 %	90.0 %
Specificity	87.5 %	75.0 %
Positive Predictive Value	93.7 %	90.0 %
Negative Predictive Value	58.3 %	75.0 %
Positive likelihood ratio	6.0	2.5
Negative likelihood ratio	0.289	0.13
Diagnostic Accuracy	78.6 %	85.7 %

DISCUSSION

Present study was carried out in the Department of Obstetrics and Gynecology of Chattogram Medical College Hospital. In this study 90 Visual Inspection with Acetic Acid (VIA) positive cases were the candidates of Pap's test and HPV DNA testing. All underwent colposcopic evaluation. The positive colposcopic cases were selected for biopsy and subsequent histopathology. Regarding different socio-demographic profiles of the study cases, most of the patients (57.8%) were in age group 30- 40 years, most of them were from average socio-economic group (66.7%). Mean age of the patients were 39.3 years. The mean duration of marital life is 21 years and the age at first intercourse was 18.18 years. Cervical lesions are common above 35-40 years so present age distribution of the designed study patients are as expected.

A study carried in India showed that the risk of HPV infection was higher in women aged 25 to 34 years (Odds ratio 1.11)¹³. In another study among 70 cases of CIN that mean age was 34.9 years¹⁴. So this figure is near similar to the present study regarding the age distribution of the patients selected in this study and regarding socioeconomic condition most were from average which is also supported by a study done in Bangladesh¹⁴.

Regarding Pap's smear analysis where NILM were found among 57.8%. Inflammatory lesion was found in 22.2%, HSIL and LSIL were obtained in 15.6% and 4.4% respectively. Total 18 (20.0%) positive case of Pap's smear was found in the study. Regarding HPV-DNA analysis where 24(26.7%) cases were found positive and 66(73.3%) were found negative.

A study revealed that women with negative cytology and a positive test for oncogenic HPV-DNA had an incidence of 16.8% for more severe, 6.4% for LSIL or more severe and 2.2%

for HSIL or more severe¹⁵. By comparison, women with negative baseline tests and a negative test for oncogenic HPV DNA at enrollment had a crude cumulative incidence for ASC or more severe of 4.2%, for LSIL or more severe of 1.1%, and for HSIL or more severe of 0.3%. So findings are consistent with our present study also.

Present study showed that Pap's smear, as a screening test, is very different from HR HPV-DNA detecting test for precancerous cervical lesion. By statistical analysis Pap's smear is clearly more specific than HPV-DNA testing but sensitivity is higher in HPV DNA testing than Pap's smear results. In practice, when these tests are used alone as a single test in mass screening for cervical cancer, results will not be satisfactory. Pap's smear misses some of the cancerous or precancerous cases, while HPV-DNA test will produce a lot of false positive cases.

In this study the amount of HPV-DNA positivity did not correlate strongly enough with the severity of the lesion to be used in practice, as shown in some other articles. Regarding validity analysis of different tests in this study that were performed for cervical precancerous conditions taking histopathology results as a gold standard, sensitivity of Pap's smear was found lower (75.0%) than the HPV-DNA(90.0%) whereas specificity of Pap's Smear was higher(87.5%) then the specificity of HPV-DNA(75.0%).

The sensitivity of the Pap's smear in this study is not that high but it correlates well with the review analysis of who reported that the sensitivity of Pap's smear ranged from 44 to 78%¹⁶. In a pooled analysis of five studies in India involving 22,663 women, the sensitivity of Pap's smear varied from 36.5% to 78%. However reduced sensitivity of Pap's smear may be related to sampling error or interpretive error. The sampling error is the single most important factor that includes specimen procurement and processing steps. The fact that most false negative diagnosis are attributed to sampling also indicates that the greatest improvement may be gained by addressing this phase.

The high sensitivity of HPV-DNA in the present study are closely correlated with a study¹⁷. Further lower sensitivity was also reported by another study¹⁸. Also we found the positive likelihood ratio of Pap's smear and HPV-DNA were 6.0 and 2.5 and negative likelihood ratio of the both test were 0.289 and 0.13 respectively. The results clearly signifies the superiority of HPV-DNA over Pap's smear.

There is a fairly good consistency of this study if compared with results of a review including several cross sectional studies using a double-testing design and meta-analysis on triage studies⁸. Follow up activity after the screening visits (e.g. a new test after one year for those with HPV positive and cytology negative) might have revealed some more cancer or pre-cancer cases. Irrespective of the limitations of the cross sectional design used in this study, one can argue that a posterior cytological testing in connection with a positive HPV result, combined with the longitudinal follow up, might lead to optimal sensitivity and also specificity in the screening activity.

Cervical cancer develops slowly, as a rule in 10–15 years via precancers. Taking advantage of this, organized cervical cancer screening and its preventive effect is based on repeated Pap's tests with 3–5 year intervals, although one single Pap's test already gives increased protection against cervical cancer when Pap's smear test is repeated in a screening program, the sensitivity is increasing up to 80–90%⁵.

In this study we found that the use of HR HPV DNA test alone, using the standard positivity, have the better result in terms of sensitivity, but with much higher costs. The fact that HPV test has high sensitivity and low specificity and Pap's smear low sensitivity and high specificity could be used to support each other.

From this study it is already known that Pap's smear cytology-based screening is not well-organized enough in Bangladesh. Those who are being detected by the screening process are the symptomatic ones. The Pap's test is done as a part of investigations related to the management of these patients. Therefore, facilities for cytological screening should be extended up to the primary health care level. Our study signifies the importance of HPV-DNA testing and as it was found superior over Pap's smear in terms of sensitivity and specificity so if possible, HPV testing should be included in the routine screening procedure of cervical lesion evaluation. All the women who were reported as LSIL / HSIL in our study were counseled and advised for colposcopic biopsy and histopathology. Our's is a hospital-based study and an advanced study under a well-organized screening system, with a large number of cases, is in demand, to reveal the exact statistics of premalignant and malignant cervical lesions, in Bangladesh.

LIMITATIONS

The authors encountered few limitations in the study including small sample size and data were obtained from single study center that might not be useful for generalization. There was also absence of long term follow up of the patients.

CONCLUSION

We conclude that in our resource limited country, lack of community participation and noncompliance remain the major obstacles to successful reduction in cervical cancer mortality. HPV-DNA testing was both more sensitive and specific near to Pap's cytology. So the use of a less invasive and more user friendly primary screening strategy like HPV-DNA testing, may be required to achieve the coverage necessary for effective reduction in cervical cancer mortality.

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DISCLOSURE

All authors hereby declare no competing interest.

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