RESEARCH ARTICLE

Role of the HPV DNA Test in Follow-up of Treated Cervical Intraepithelial Neoplasia in Bangladesh

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Abstract

Background: Cervical cancer is a major public health problem in Bangladesh. Persistence of high risk human papillomavirus (HRHPV) influences the progression of the disease, with an important role in follow-up for cervical intraepithelial neoplasia (CIN). Objective: To establish application of high risk HPV DNA test in the follow-up of women after treatment of CIN. Materials and Methods: This cross-sectional and hospital based study was carried out among 145 CIN treated women during the previous six months to three years at the colposcopy clinic of Bangabandhu Sheikh Mujib Medical University, Dhaka, between January 2011 and June 2012. Pap smear and HPV samples were collected and colposcopy was performed to find out the persistence of the disease. Cervical samples obtained were tested for HPV DNA using the Hybrid Capture II (HC-II) test. A cervical biopsy was collected whenever necessary. The results were compared to assess the efficacy of different methods during follow up such as Pap smear, HPV test and colposcopy. Results: Mean age of the recruited women (n=145) was 33.6 (± 7.6), mean age of marriage was 16.8 (±2.9) and mean age of 1st delivery was 18.8 (±3.5) years. More than half had high grade CIN before treatment and 115 (79.3%) women were managed by LEEP and 20.7% were managed by cold coagulation. Among the 145 treated women, 139 were negative for HPV DNA and six of them (4.1%) were HPV positive. Sensitivity of Pap smear (40.0) and HPV DNA test (40.0) was poor, but specificity was quite satisfactory (>93.0) for all the tests. Conclusions: The high risk HPV DNA test can be an effective method of identifying residual disease. It can be added to colposcopy and this should be applied to all treated women attending for their first or second post-treatment follow-up visit at 6 months to one year, irrespective of the grade of treated CIN.

Keywords: HPV DNA test - follow-up - treated CIN - Bangladesh

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Introduction

In Bangladesh, the incidence of cervical cancer is about 17,686 and around 10,364 women die from cervical cancer each year (Ferlay et al., 2008). Hospital based data revealed that cervical cancer constitutes 22-29% of the female cancer in Bangladesh (Akhter et al., 1998). Therefore screening and education intereventions are necessary (Nessa et al., 2013a; 2013b).

Cervical cancer is a slowly progressing tumour and its natural history is reasonably well understood. Diagnosis and treatment of cervical intraepithelial neoplasia (CIN) through population-based screening programs has lead to 50-80% reduction in deaths from cervical cancer in various developed countries (IARC, 2004). Bangladesh has a comprehensive health infrastructure which offers the feasibility of introducing screening programmes. The government of Bangladesh is developing a nationwide cervical cancer screening programme through a less expensive method. Visual inspection of Cervix with Acetic Acid (VIA) is performed at all Maternal and Child Welfare Centres (MCWCs), District Hospitals (DHs), Medical College Hospitals (MCHs) and Bangabandhu Sheikh Mujib Medical University (BSMMU) by trained Family Welfare Visitors (FWVs), Senior Staff Nurses (SSNs) and Doctors (Ahmed et al., 2008; Nessa et al., 2010). These trained persons offer VIA technique to detect the precancerous conditions or initial stages of cervical cancer among women 30 years and above visiting the mentioned centres of all the districts of Bangladesh. VIA test is considered positive when an aceto-white area is identified within the transformation zone. Screening test positive women are referred to BSMMU and various government medical college hospitals for colposcopic evaluation and management. About 50% of these referred women attend the colposcopy clinic of BSMMU to avail evaluation and management of CIN. The value of any treatment for CIN is measured by its success in

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curing the disease and follow-up of treated women is necessary to assess the cure rate. At colposcopy clinic of BSMMU, women are treated by cold coagulation or loop electrosurgical excision procedure (LEEP). After treatment these women are offered colposcopy for follow up every year for subsequent three years. Traditionally post-treatment follow up of women is performed by Pap smear and colposcopy. Though Pap smear is an effective way of follow-up in developed countries, in developing countries this method is associated with a very high false-negative report due to sampling errors (presence of blood, mucus and inflammation and difference of collection device), sample preparation errors (fixing, staining etc) etc (Martin-Hirsch et al., 1999). Apart from that this method needs an appropriate health system and set-up.

Recently the role of HPV DNA test is becoming popular for follow-up of women. As HRHPV (high risk HPV) infection plays substantial role in developing cervical cancer, HRHPV DNA detection plays crucial role in follow-up. Persistence of certain risk groups of HRHPV influence the progress of the disease, presence of this virus may have important role in follow-up of treated CIN. HRHPV infection associated with CIN clears gradually after successful treatment. In one study, clearance occurred within 6 months after treatment in most patients and few additional clearances took place after 12 months. Clearance rates were significantly slower in patients with HPV DNA loads >500 RLU/PC (Young et al., 2010). Therefore, HPV DNA test can be used for follow-up of CIN patients after treatment. Colposcopic examination can be avoided or less frequently done among post-treatment HRHPV negative cases. In this study hybrid capture II (HC II) was used for detection of HRHPV DNA in the cervical samples from 145 women who received treatment six months to three years before the collection of samples. All these women also had Pap smear and colposcopy during the same follow-up visit. The objective of the study was to the role of HRHPV DNA test in the follow-up of CIN treated women.

Materials and Methods

This cross-sectional and hospital based study was carried out at the colposcopy clinic of Bangabandhu Sheikh Mujib Medical University (BSMMU) between January 2011 and June 2012. The women with history of treatment of high-grade CIN during the last six months to three years were recruited after written consent. After counseling and motivating the study population, data were collected from treated cases of CIN during follow-up visit at colposcopy clinic by face to face interview. Following initial interview, a Pap smear was collected from the cervix using ayllesbury spatulla and cytobrush. Then DNA specimen was collected prior to application of acetic acid and colposcopy. Cervical Sampler was used for collection and transport of cervical specimens for HPV DNA. Excess mucus was removed from the cervical os and surrounding ectocervix using cotton swab. The brush was inserted 1-1.5 cm into the cervical os until the largest outer bristles of the brush touched the ectocervix. The brush was rotated three full turns in a counter clockwise direction. Care was taken so that the brush was not completely inserted into the cervical canal. The specimens were stored at -20ºC upon receipt at the laboratory and tested within three months in batches. A cervical biopsy was collected whenever necessary. Cytology, virology and histology examinations were performed in the respective departments of BSMMU. In this study Hybrid Capture II (HC II) was used for HRHPV DNA detection. The Digene HPV Test using HC II technology is a linear signal amplified hybridization antibody capture micro-plate assay. Qualitative detection of thirteen types (16/18/31/33/35/39/45/51/52/58/59/68) of high risk HPV DNA in cervical specimens were carried out using chemiluminescent detection. For detection of high-grade dysplasia, the sensitivity range of this test has been recorded as 80-90% and specificity has ranged from 57-89% (Manos et al., 1999; Cuzick et al., 2000).An RLU measurement equal to or greater than the cut off value indicates the presence of target HPV DNA sequences in the specimen. Patient RLU/ Known Positive RLU cut off at Ratio between 0 to 1.2 is considered as negative and ratio above 1.2 is Positive for Test. The lower limit of HPV DNA detection of the Digene HPV Test using the Hybrid Capture 2 System is 1pg/ml. This corresponds to 113,636 genomes/mL or 5682 genomes per assay.

In this study colposcopy result was considered as 'gold standard' to determine the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) of VIA test, Pap smear and HPV DNA test in the post-treatment follow up cases. In case of colposcopy, all CIN cases regarded as positive and others considered as negative. In case of VIA, unsatisfactory findings considered as negative. In case of Pap smear, high grade lesions and CIN cases measured as positive, others (normal, ASCUS and unsatisfactory) were considered as negative and unavailable reports were excluded to calculate the findings. RLU value 0- 1.2 considered as negative and RLU value >1.2 considered as positive HPV DNA test.

Results

One hundred forty five women were enrolled in the study. These women had collection of Pap smears, HPV samples and colposcopy examinations. Table 1 showed the socio-economic and demographic characteristics of the women. Mean age of the women was 33.59 (±7.69) years and their age ranged from 19 to 51 years. Majority of the women were housewives and belonged to middle income group. A good number of them had more than 5

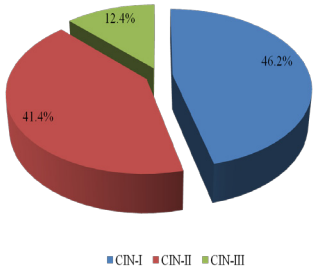

Figure 1. Previous Histological Diagnosis of Women (n=145)

years of schooling.

Table 2 showed the reproductive characteristics of the women. Mean age of marriage of women was 16.83 (±2.9) and their mean age of 1st delivery was18.79 (±3.5) years. The Mean parity of the women was 2.42 (±1.2).

Figure 1 showed previous histological diagnosis of the recruited women. More than half of them (53.8%) had high grade CIN before treatment.

Among the 145 women, 115 (79.3%) women were managed by LEEP and 20.7% were managed by cold coagulation (Table 3). Majority of the high grade CIN were treated by LEEP.

At follow up, among the 145 treated women, 139 were negative for high risk HPV DNA and six of them (4.1%) were positive (Table 4). Majority of the high risk HPV DNA negative cases had normal colposcopy findings and two had CIN I (RLU 1.0 and 0.2) which may be related to low risk HPV infection. Only one woman with colposcopy diagnosed CIN II was HPV negative and her repeat LEEP histology revealed CIN I.

Of the six women with HPV-positive report, three women (RLU 2.1) had colposcopy diagnosed CIN I, one (RLU 3.9) had CIN II and two (RLU 82.4, 24.7) had normal colposcopy findings (Table 4). All these findings indicate that high risk HPV DNA test is effective to identify residual diseases and it can help to identify residual diseases which are missed or underdiagnosed by colposcope.

Among the five colposcopy diagnosed CIN I, only one woman had positive report by Pap smear and one of the two colposcopy diagnosed CIN II, one had abnormal Pap smear (Table 5).

Considering colposcopy as gold standard the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of VIA test, Pap smear and HPV DNA test were calculated. Sensitivity of Pap smear (40.0) and HPV DNA test (40.0) was poorer than VIA test (60.0), but specificity was quite satisfactory (>93.0) for all the tests (Table 6). The specificity of HPV DNA test was 97.14.

Table 1. Socio Economic and Demographic Characteristics of Women (n=145)

Characteristics	Categories	Number (%)
Age	0-25 years of age	17 (11.7)
	26-30 years of age	45 (31.0)
	31-35 years of age	36 (24.8)
	36-40 years of age	23 (15.9)
	41-45 years of age	11 (7.6)
	46 & above	13 (9.0)
Religions	Islam	135 (93.1)
	Hindu	10 (6.9)
Women's Education	No formal education	19 (13.1)
	1-5 years of schooling	27 (18.6)
	5-10 years of schooling	50 (34.5)
	More than 10 years of education	49 (33.8)
Women's Occupation	Housewife	131 (90.3)
	Service Provider	14 (9.7)
Monthly family income	Poor (less than Tk. 5000)	11 (7.6)
	Lower middle class (Tk. 5001-10000)	67 (46.2)
	Middle class (Tk. 10001-30000)	55 (37.9)
	Upper class (more than Tk. 30001)	12 (8.3)
Husband's Occupation	Business	41 (28.3)
	Non-govt. service	36 (24.8)
	Others	23 (15.9)
	Govt. Service	17 (11.7)
	Farmer	11 (7.6)
	Day Labour	9 (6.2)
	Husband works abroad	8 (5.5)
Husband's Education	No formal education	20 (13.8)
	1-5 years of schooling	19 (13.1)
	5-10 years of schooling	34 (23.4)
	More than 10 years of education	72 (49.7)

Table 2. Reproductive Characteristics of Women (n=145)

Characteristics	Categories	Number (%)
Age of marriage	<18 years	109 (75.2)
	19-20 years	21 (14.5)
	21 and above	15 (10.3)
Age of delivery	<18 years	71 (49.0)
	19-20 years	32 (22.1)
	21-25 years	38 (26.2)
	>25 years	4 (2.8)
Number of marriage of women	One	140 (96.6)
	Two	5 (3.4)
Number of marriage of husband	One	134 (92.4)
	Two	11 (7.6)
Parity	0-2	89 (61.4)
	3-4	48 (33.1)
	5-6	8 (5.5)

Table 3. Mode of Treatment of Different Histologically Diagnosed Cases of CIN (n=145)

Previous histological diagnosis	Mode of Treatment		Total
	Cold Coagulation	LEEP	
CIN-I	22	45	67
CIN-II	8	52	60
CIN-III	0	18	18
Total	30	115	145

Table 4. Comparison of High Risk HPV DNA Value and Colposcopy Diagnosed CIN

HPV DNA Value	Colposcopy report				Total
	Normal	CIN-I	CIN-II	Unsatisfactory	
High risk HPV DNA Negative (0-1.2)	134	2	1	2	139 (95.9%)
High risk HPV DNA Positive (≥1.2)	2	3	1	0	6 (4.1%)
Total	136	5	2	2	145 (100.0)

Table 5. Comparison between Colposcopy Diagnosed CIN and Pap smear Report

Colposcopy report	Pap Smear Report						Total
	Normal	ASCUS	Low grade lesion	High grade lesion	Not available	Unsatisfactory	
Normal	121	1	1	0	12	1	136
CIN-I	4	0	0	0	1	0	5
CIN-II	1	0	0	1	0	0	2
Unsatisfactory	2	0	0	0	0	0	2
Total	128	1	1	1	13	1	145

Table 6. Sensitivity, Specificity, Positive Predictive value (PPV) and Negative Predictive value (NPV) of VIA test, Pap smear and HPV DNA test

Test of Validity	VIA	Pap smear	HPV DNA
Sensitivity	60.0	40.0	40.0
Specificity	93.6	100.0	97.1
PPV	25.0	100.0	33.3
NPV	93.6	100.0	97.1

Discussion

High risk HPV DNA test has immense potential to identify women at greater risk of disease progression. It can identify the women with risk of residual disease in the cervices. Among the 145 treated women, majority of the cervices (95.9%) of women revealed absence of high risk HPV DNA prove that treatment of pre-cancer both by LEEP and cold coagulator is effective to eradicate the disease in majority of the cases. It also proved that High risk HPV DNA test has the capability to identify women at risk of disease persistence. Several studies confirmed a similar finding and revealed that after successful CIN III treatment HPV DNA is no longer detectable even by highly sensitive methods (Kjellberg et al., 2000; Elfgren et al., 2002). Kjellberg et al found only three women were HPV DNA positive 3 years after laser conization of 82 initially HPV DNA positive women with CIN I-III (Kjellberg et al., 2000).

Our study found that majority of the high risk HPV DNA negative cases following treatment had normal colposcopy findings and two women had Colposcopy diagnosed CIN I and one with colposcopy diagnosed CIN II with LEEP histology diagnosed CIN I proved that HR HPV DNA test did not fail to pick up any high grade lesion. All these findings reflect that high risk HPV DNA test is an acceptable method of post-treatment surveillance of pre-cancer of cervix.

One of the objectives of the present study was to investigate whether high risk HPV can be cleared after treatment. The Mean interval between treatment and follow-up of the disease was 16.2±9.9 months and in majority of the cases (95.9%) the disease was cleared by 7-25 months. In two studies by Bollen et al, 88-90% of women treated for CIN had cleared their initial HPV infection at 1 year after treatment (Bollen et al., 1996; Bollen et al., 1997). The same studies also proved that persistent infection with high risk HPV virus following treatment has been associated with increased risk of recurrent high grade CIN and these women require increased monitoring. The present study did not have the scope of measuring high risk HPV DNA before initiation of treatment. However it showed association of similar rate of clearance of the disease with absence of high risk HPV DNA following treatment. Of the six women (4.1%) with high risk HPV-positive report, two had colposcopy diagnosed CIN I, two had CIN II and two women had normal colposcopy findings. This indicates that high risk HPV DNA test can identify the cases of treatment failure effectively and it is superior to colposcopy. Moreover if indicates that high risk HPV positive women with normal Colposcopy findings are still in risk of the disease and need further evaluation and long time follow-up. Other study also established that clinical HPV DNA testing is more sensitive for the detection of current and future cervical pre-cancer and cervical cancer (Philip et al., 2012).

One study concluded that ablative or excision techniques for the treatment of cervical cancer precursors are generally effective, with cure rates up to 95 per cent. In addition, treated women remain at increased risk of cervical cancer for at least 8 years compared to the general female population (Soutter et al., 1997). In this study among six high risk HPV positive women, one woman with high RLU level at follow-up revealed carcinoma in situ in both LEEP specimens during initial and follow-up treatment. Therefore HPV DNA testing may be useful for monitoring the treated women. Evidence suggested that all women treated of CIN should have an HPV test six months following treatment combined with cervical cytology and HPV test 12-18 months following treatment. If all three of these tests are negative the patient can be returned to routine three or (for those over 49) five yearly testing (Coupe et al., 2007).

One study with long term follow-up of 11,085 women in the multicentre HART study in the UK revealed that of 9247 women with both cytology and HPV negative results at the baseline, only 9 (0.2%) had CIN2+ identified during the subsequent five years of follow-up (Mesher et al., 2010).

Time-honored protocols involve follow-up with cytology tests for the early detection of recurrence of high grade CIN. In this study, the sensitivity of Pap smear was 40% and specificity was 100%. However, during this study all the Pap smears were collected by cytobrush and combination of high risk HPV genotypes and cytology testing has the potential for improving the efficiency and effectiveness of monitoring women after treatment of CIN. However the sensitivity of detection of CIN by Pap was low and probably therefore not promising method of follow-up after treatment in developing countries likes Bangladesh.

In this study HPV DNA clearance was observed both in the LEEP and cold coagulation treated group and the findings suggested that treatment by both LEEP and cold coagulation were effective treatment methods. It was seen that all the samples of cold coagulation treated group cleared out high risk HPV DNA and six samples of LEEP treated group remained high risk HPV DNA positive. This was probably related to physician's preference of treatment of more high grade cases by LEEP.

Several studies also revealed that HPV testing using HC II was sensitive test of cure and was superior to either cytology or colposcopy (Nobbenhuis et al., 2001; Paraskevaidis et al., 2004; Kitchener et al., 2008). More studies in Korea and Italy discovered that HPV testing was the most sensitive mean of identifying persistence or early relapse and is therefore capable of optimizing follow-up after the treatment of high-grade CIN (Jeong et al., 2009; Kang et al., 2010; Ribaldone et al., 2010). Moreover HPV test improves the sensitivity of a post treatment test allowing the recognition of early treatment failures even in the presence of normal cytology (Agorastos T et al. 2010).

In this study majority of the women became disease

free after treatment and we suggest that the high risk HPV test was useful to identify this fact. Again few women became disease free, at least temporarily, but did not clear the HPV infection after treatment and more follow-up was needed.

In Bangladesh, following treatment, women are usually followed up by colposcopy only. From this study, we can suggest that women with normal colposcopy and negative HR-HPV test should be invited for their next colposcopy test in three years irrespective of their age. At the three year follow-up, if the colposcopy result is negative, women should revert to their routine screening protocol (i.e. VIA test every three years). During follow-up women with positive HR-HPV test should be referred for colposcopy. The main additional costs associated with implementing HPV test at follow-up will be taka 2000 ($ 25) at BSMMU to taka 3000 ($38) at private diagnostic centers.

This study concluded that high risk HPV DNA test can be an effective method of identifying residual disease. It can be added to colposcopy and this should be applied to all treated women attending for their first or second post-treatment follow-up visit at 6 months to one year, irrespective of the grade of treated CIN.

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