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Evaluation of HPV-DNA Test in Detection of Precancerous and Cancerous Lesions of Cervix.

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Research Article

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ABSTRACT

The knowledge that cervical neoplasia are caused by Human Papillomavirus (HPV) infection has led to the evaluation of its role in screening of cervical neoplasia. This study was carried out to evaluate the accuracy of HPV-DNA test in diagnosis of precancerous and cancerous lesions of cervix in relation to histopathology. Total no of 115 eligible women were included in this study. After recording relevant data cervix was examined on naked eye by cuscos speculum. Paps smear collection and VIA tests were done concurrently. Colposcopic examination was done who were positive in screening tests. In addition, subjects with grossly abnormal cervix even with negative in screening tests were also referred for colposcopy. Samples for HPV DNA were taken from the patients referred for colposcopy and biopsies were done in the same patients. Those with CIN I or worse lesions diagnosed by histology were considered as true positive. The study results showed the test parameters for VIA were sensitivity of 94.11%, specificity of 57.57%, positive predictive value of 12.20%, and negative predictive value of 99.70%. The test parameters for Pap smear were sensitivity of 64.71%, specificity of 94.29%, positive predictive value of 51.70% and negative predictive value of 99.80%. The test parameters for HPV DNA test were sensitivity of 82.35% and specificity of 84.85%, positive predictive value of 73.68% and negative predictive value of 90.32%. VIA and HPV-DNA tests detected all cases of high grade lesions (CIN II & III) and carcinoma. This study was that VIA is superior to Pap smear cytology and HPV-DNA test in sensitivity, that is VIA can more accurately identify the CIN/ cancer patients, On the other and Pap smear is superior to VIA and HPV-DNA test in specificity that it can more accurately identify the truly well people and HPV-DNA has strong association in high grade lesions of the cervix.

INTRODUCTION

Cervical cancer is the second most common cancer that affects women and may constitute up to 25% of all female cancers in developing countries [1]. It affects nearly half a million women each year worldwide, claiming a quarter of a million lives i.e., 50% mortality rate [2]. Bangladesh and India have an annual incidence of cervical cancer 11956 and 125952 respectively [3]. It constitutes about 24.6% among total female cancer in Bangladesh [4]. There are now ample evidences that persistent infection by a 'high risk' subset of Human Papilloma virus (HPV) is the single most important risk factor of cervical cancer [5,6,7]. The HPV family of viruses contains more than 100 types; which are referred to by number. Approximately 40 types of HPV are known as genital HPV since they affect the genital area [8]. Genital HPV infections are frequently asymptomatic and resolve without causing disease. However, some HPVs cause benign skin warts, or papillomas, for which the virus family is named and certain HPV

infections can cause cervical cancer [9]. HPV types associated with cancer are called oncogenic or 'high risk' types; 13 have been recognized by International Agency for Research on Cancer (IARC) [10]. The most common oncogenic HPV type in squamous cervical cancer is HPV 16, 18 found in more than 80% of the cases [11]. HPV types that do not cause cancer are termed 'low risk'. Two of these 'low risk' types cause genital warts (HPV 6 and 11). Genital HPV is so common that it can almost be considered a normal consequence of having sex. Estimates suggest that between 50% and 79% of all women who have had sexual intercourse have a lifetime risk of becoming infected with one or more of the sexually transmitted HPV types [12]. Often the infection is transient and it is only when it becomes persistent may lead to Cervical Intraepithelial Neoplasia (CIN).

It is not certain why in some women, persistent HPV infection causes more serious problems than in others. There are, however, several identified co-factors which increase the risk of cervical cancer. These include: women experiencing first intercourse at an early age, having multiple sexual partners, or having intercourse with a male partner who has had multiple sexual partners, having more than four full term pregnancies, smoking habit and immune suppression. There is limited evidence to suggest that four or more years of oral contraceptive pill use may have a role [13]. The incidence of cervical cancer decreased significantly since the 1960 [14]. Much credit for these dramatic gains belongs to the effectiveness of screening by cervical cytology and to the accessibility of the cervix to colposcopy and biopsy in detecting cervical precancers. Screening for cervical cancer is based on the theory that all invasive cancers are preceded by a series of precursors known as CIN that can be detected by cervical cytology. Cervical cancer is usually by a long phase of cytological changes and takes a long period of 10-15 years before the invasive cancer develops [15].

The first screening method for cervical cancer i.e., Pap smear technique relies on a microscopic examination of cervical cells collected during the cervical smear procedure. This method allows detection of cellular changes indicating the possible genesis of cervical cancer. The VIA is another screening test which involves swabbing the cervix with 3% to 5% acetic acid solution and examination of cervix in good light. Abnormal cells temporarily turn white and reveal aceto-white epithelium on the cervix. The HPV detection is an objective quality assurance benchmark for cervical cytology [16]. Recently, a hybrid capture technique has been developed to document the presence of the virus in liquid samples obtained from the female genital tract [17]. Several studies had been carried out in different countries of the world on Pap smear, VIA and HPV-DNA for early detection of cervical carcinoma. This study was also carried out to see the accuracies of pap smear, VIA and HPV-DNA test in diagnosis of precancerous and cancerous lesions of cervix in relation to histopathology.

MATERIALS AND METHODS

This prospective study was carried out in the Department of Obstetrics and Gynaecology in collaboration with the department of Pathology, Rajshahi Medical College, Rajshahi; Bangladesh, during the period from July 2006 to June 2008. A total number of 115 patients were selected from the patients attending the Gynae Out Patient Department (OPD) according to enrolment criteria. Married women above 30 years of age or women having marital life more than 10 years attending Gynae OPD for any Gynecological problems were referred to VIA Centre. Unmarried women, women who were currently pregnant or who had a history of abnormal cytology, previous treatment for CIN or cancer, were excluded from the study.

Methods of data collection

Data were collected from the enrolled patients by using a questionnaire. After recording clinical history, cervix was examined on naked eye by cuscos speculum. Paps smear collection and VIA tests were done concurrently. Colposcopic examination was done who were positive in screening tests. In addition, subjects with grossly abnormal cervix even with negative in screening tests were also referred for colposcopy. Samples for HPV-DNA were taken from the patients referred for colposcopy and biopsies were done in the same patients. Clinical history, physical findings, Pap smear findings, VIA findings, DNA tests and histological findings were recorded in the pre-designed patient's profile made for the study.

Technique of Pap smear preparation

A clean dry glass slide was numbered. The small end of the wooden Ayre's spatula was placed through the external os high into the canal. The spatula was then rotated clockwise at 360^0 angle thoroughly for scraping the entire cervical os. The collected samples were spread on two-thirds of clean glass slides, which were immediately dipped into Coplin jar containing fixative (95% ethyl alcohol) for at least 30 minutes. Then the smears were stained by modified Papanicolaou staining method for cytological diagnosis. Cytology was considered positive if any of the following lesions were reported: dysplasia of any grade, carcinoma in situ and invasive cancer. Pap smears were evaluated and diagnosed using the Bethesda system.

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Technique of VIA

After obtaining the specimen for cytology, 5% acetic acid was applied to the cervix for 1 minute and inspection was done to see any acetowhite area around squamocolumner junction (SCJ) or in transitional zone (TZ). A normal cervix had no white lesions. A low- grade CIN showed pale white lesions that might or might not abut the SCJ. Well defined, dense, acetowhite areas with regular or irregular margins close to SCJ or in TZ or dense, aceto-whitening of ulcer-oproliferative growth on the cervix were regarded as high grade CIN.

Technique of HPV-DNA sample collection

The samples for HPV DNA testing were obtained by washing the ectocervix, endocervical canal using a special brush by rotating anticlockwise for 3 times and place into HPV collection kit and stored at -20^0C until further processing. HPV DNA detection was carried out using a commercially available kit; the Hybrid captures II (Digene Diagnosis HPV Test-IVT) as per the instructions of the manufacturer protocol. The reference investigation (gold standard) for evaluating the accuracy of tests in detecting true positive lesions was histology. Women with a final diagnosis of CIN or carcinoma in situ were considered as true positive cases for the estimation of sensitivity, specificity and predictive values of the screening tests. The estimates for sensitivity, specificity and predictive values were calculated using standard formulae for these tests, using a 2×2 contingency table.

RESULTS AND OBSERVATIONS

Out of 115 patients screened, 30 (26.09%) were VIA positive, 85 (73.91%) were VIA negative. Pap smear was positive in 13 (11.31%) cases and negative in 102 (88.69%) cases. Finding of VIA positive and Pap smear positive cases were evaluated colposcopically and final diagnosis was made on histopathological reports. Samples for HPV DNA were taken from the patients referred for colposcopy and biopsies were done of the same patients. Findings of VIA, Paps, HPV DNA tests and histopathology reports were shown in Table 1. In Pap smear cytology, 98 (85.22%) cases were diagnosed as “Inflammatory/Negative for intraepithelial lesions or malignancy”. Again, 1 (0.87%) was diagnosed as ‘atypical squamous cell of undetermined significant’ (ASCUS), 6 (5.22%) cases were as Low-grade squamous intraepithelial lesions (LSIL), 4(3.48%) cases were High-grade squamous intraepithelial lesions (HSIL). 2 (1.74%) cases were found to be squamous cell carcinoma. Remaining 4(3.47%) had unsatisfactory results which included in other diagnostic findings (Table 2). On histopathological examinations of 50 biopsy specimens, 33 (66%) cases were diagnosed as inflammatory or chronic cervicitis, 9(18%) cases were diagnosed as CIN-I, 1(2%) cases were diagnosed as CIN-II, 4(8%) cases were diagnosed as CIN-III, 3(6%) cases were diagnosed as invasive squamous cell carcinoma (Table 3).

Table 1: Findings of VIA, Paps, HPV DNA tests and histopathology

Tests	Positive (%)	Negative (%)	No. of patients
VIA	30 (26.09)	85 (73.91)	115
Pap test	13(11.31)	102(88.69)	115
DNA	19(38)	31(62)	50
Histopathology	17 (34)	33 (66)	50

Figure 2: Pap smear cytological diagnosis of the study patients (n=115)

Pap smear findings	Frequency	Percentage (%)
Inflammatory	98	85.22
ASCUS-H	1	0.87
LSIL	6	5.22
HSIL	4	3.48
Carcinoma	2	1.74
Unsatisfactory	4	3.48

Table 3: Histopathological findings of screening positive cases (n=50)

Histopathological findings	Frequency	Percentage (%)
Inflammatory	33	66
CIN-I	09	18
CIN-II	01	2
CIN-III	04	8
Carcinoma	03	6

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VIA tests and its relation to histopathology

Of the total 50 cases in which biopsy were done, VIA tests were positive in 30 cases and negative in 20 cases. Out of 33 histologically diagnosed cases of chronic cervicitis, 14(42.42%) were VIA positive and 19(57.58%) were VIA negative. Out of 9 CIN-I cases, 8(88.89%) cases were VIA positive and 1(11.11%) was VIA negative. All 5(100%) CIN-II/III cases and all 3(100%) malignant cases were VIA positive (Table 4).

Table 4: VIA and its relation to Histopathology (n=50)

Histological diagnosis	No. of patients	VIA test results	
		Positive (%)	Negative (%)
Chronic cervicitis	33	14(42.42)	19(57.58)
CIN I	9	8(88.89)	1(11.11)
CIN II/III	5	5(100)	-
Squamous cell carcinoma	3	3(100)	-
Total	50	30	20

False positive = 14 (Disease negative but test positive)
False negative = 1 (Disease positive but test negative)
True positive = 16 (Those who are both test positive and disease positive)
True negative = 19 (Those who are both test negative and disease negative)

Pap smear cytology and its relation of Histopathology

On the histologic basis, among the 33 cases of chronic cervicitis, 31 (96%) cases were correctly diagnosed cytologically as inflammatory cytology or negative for intraepithelial lesions. Out of the 9 CIN-I lesions, 4(44.4%) cases were diagnosed as LSIL and out of 5 CIN-II/III 4(80%) cases were diagnosed correctly as HSIL by cytology. Among 3 the cases of squamous cell carcinoma, 2 were correctly diagnosed by Pap smear cytology (Table 5).

Table 5: Pap smear cytology and its relation of Histopathology (n=50)

Histological diagnosis	No. of patients	Pap smear cytological diagnosis				
		Inflammatory cytology	ASCUS	LSIL/CIN-I	HSIL/ CIN-II-III	Sq. cell carcinoma
Chronic cervicitis	33	31(96 %)	1(3.2%)	1 (3.2%)	-	-
CIN-I	9	4(44.4%)	-	5(55.6%)	-	-
CIN-II/III	5	1 (20%)	-	-	4 (80%	-
Invasive Sq.cell carcinoma	3	1(33.3%)	-	-	-	2 (66.7%)
Total	50	37	1	6	4	2

False positive = 2 (Disease negative but test positive)
False negative = 6 (Disease positive but test negative)
True positive = 11 (Those who are both test positive and disease positive)
True negative = 31(Those who are both test negative and disease negative)

HPV-DNA test and its relation of Histopathology

Among 50 cases, 19 (38%) were HPVDNA positive and 31 (62%) were HPV DNA negative. Histologically, 5(16.13%) cases of chronic cervicitis, 6(66.67%) cases of CIN-I and all cases of CIN-II/III, squamous cell carcinoma showed HPV DNA test positive (Table 6). The statistical evaluation of this study was based on histologically confirmed 50 cases. In the present study, VIA test was accurate in 70% of cases with 94.1% (16 of 17) true positive, 5.9 %(1 of 17) false negative, 57.6 %(19 0f 33) true negative and 57.6 %(14 0f 33) false positive cases (Table 7). Pap smear cytology was diagnostically accurate in 84% of the cases with 64.7% (11 of 17) true positive , 35.3% (6 of 17) false negative, 93.9% (31of 33) true negative and 6% (2 of 33) false positive case. HPV DNA test was diagnostically accurate in 84.62% of the cases with 83.35%(14 of 17) true positive, 17.65%(3 of 17) false negative,84.84%(28 of 33) true negative,15.15%(5 of 33) false positive (Table 7). The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), for the HPV DNA were82.35%, 84.85%, 73.68%, 90.32%, pap smear were 64.7%, 93.94%, 84.62%, 83.78%, and for the VIA test were 94.11%, 57.57%, 53.53%,95% respectively (Table 7).

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Table 6: HPV DNA test and its relation of Histopathology (n=50)

Histopathological diagnosis	No. of patients	HPV DNA Test Results	
		Positive (%)	Negative (%)
Chronic cervicitis	33	5(15.15)	28 (84.85)
CIN I	9	6(66.67)	3 (33.33)
CIN II	1	1(100)	-
CIN III	4	4(100)	-
Sq. cell carcinoma	3	3(100)	-
Total	50	19	31

False Positive = 5 (Disease negative but test positive)
False Negative = 3 (Disease positive but test negative)
True positive = 14 (Those who are both test positive and disease positive)
True negative = 28 (Those who are both test negative and disease negative)

Table 7: Statistical analysis of VIA, Pap smear cytology and HPV DNA test

Diagnostic Methods	True positive	True negative	False positive	False negative	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
VIA	16	19	14	1	94.11	57.57	53.33	95.00	70.00
Pap	11	31	2	6	64.71	93.94	84.62	83.78	84.00
HPV	14	28	5	3	82.35	85.71	73.68	90.91	84.62

DISCUSSION

This study has addressed the test performances of three screening approaches (VIA, Pap smear and HPV DNA test) to detect cervical neoplasm. To evaluate the success of the present study; the findings were compared with observations by others. In reviewing the observations by different authors it had been seen that sensitivity of VIA ranged from 60.50% to 94.12% [18,19,20,21] and specificity ranged from 30.4% to 88.5% [22, 23]. Sensitivity of Pap smear ranged from 29.6% to 83.30% [24, 25] and specificity ranged from 69.60% to 97.90% [22, 26, 27]. Regarding HPV DNA test sensitivity ranged from 45.70% to 94.40% and specificity ranged from 69.30 % to 94.60% [20, 28, 29]. In this study, the sensitivity of VIA (94.11%) was higher than that observed from other cross sectional studies conducted in Zimbabwe (76.10%), China (70.90%), and India (88.60%) which used nursing, paramedical and medical background. However, the specificity of VIA (57.57%) was lower in our study as compared to these reports [21,22,23,24,25,26,27]. Comparing VIA with cytology Gaffikin noted that overall usefulness of VIA compares favorably with that of the Pap test [16]. VIA and HPV-DNA tests detected all cases of high grade lesions (CIN II & III) and carcinoma. The performance of cytology in detecting lesions was far from satisfactory and in reviewing the observations by different authors it had been seen that Pap smear had a wide range of sensitivities (28.20 to 83.30%) [25, 35]. The best estimates suggest that Pap test is moderately sensitive and it is likely that the frequently repeated testing in screening programs in developed countries contributed to their success despite moderate even low sensitivity [36, 37].

Though in this study the sensitivity of Pap smear (64.71%) was clearly inferior to that of VIA (94.11%), the specificity was significantly greater (93.94%) than that of VIA (57.57%). In this study the positive predictive value and negative predictive value for VIA were 53.53% and 95%. These values were also closely similar to those of Hussain [23]. The negative predictive value for VIA (95%) in this study also reflects a chance of missing CIN/cancer was 5%.The positive predictive value and negative predictive value for HPV DNA of this study were 73.68% and 90.32%. These values differ with those of Israt [24]. The positive predictive value and negative predictive value for Pap smear of this study were 84.62% and 83.78%. These values were more or less similar to those of Begum [25].

In explaining the present study it had been shown that after getting a negative Pap smear result, the probability of not having CIN/cancer was 83.78% and the chance of missing CIN/cancer was 16.22%. This rate was very high and not suitable for cancer screening. The highest sensitivity (94.11%) was found in VIA, but the rate of false positive was considerably higher, yielding a specificity of 57.57% which indicates high degree of over diagnosis. Acetowhite areas due to immature squamous metaplasia and inflammatory lesions seem to be responsible for a large number of false positive findings.

On evaluation of results of known studies including this one it is noted that VIA test is superior to Pap smear and HPV-DNA test in sensitivity that is VIA can more accurately identify the CIN/cancer patients and Pap smear is superior to VIA and HPV DNA test in specificity that is it can more accurately identify the truly well peoples and HPV-DNA has strong association in high grade lesions of the cervix. VIA and HPV-DNA tests detected all cases of high grade lesions (CIN II & III) and carcinoma. This invariably leads to high rates of referral and high rates of treatment. On the other hand, the inherent difficulty in efficiently performing the different steps in cytology

screening is the main cause of suboptimal sensitivity to detect lesions. A drawback to HPV testing is that it is more expensive ($20 to $30 per test) and time-consuming than other screening tests, and it requires a sophisticated laboratory infrastructure. None study showed that the screening tests such as Pap, VIA and HPV-DNA test separately were suitable for the diagnosis of cervical lesions.

CONCLUSION

Recent advances in our understanding of the causes and natural history of cervical neoplasia and, in particular, the establishment of the central role of HPV infection has created opportunities for the primary and secondary prevention of cervical cancer. In the future, prevention efforts will include the incorporation of HPV testing as an adjunct to or replacement for cytology-based screening programs and the use of recombinant DNA technologies for the development of prophylactic vaccines. Since more than 99% of invasive cervical cancers worldwide contain HPV, some researchers recommend that HPV testing be done together with routine cervical screening. Others suggest that routine HPV testing would cause undue alarm to carriers, more unnecessary follow-up testing and treatment. HPV testing along with cytology significantly increases the cost of screening.

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REFERENCES

1. Burd EM. Human Papillomavirus and cervical cancer. Clin Microbiol Rev. 2003;16:1-17.
2. WHO. 2006. World Health Organization, comprehensive cervical cancer control: A guide to essential practice, 15-191.
3. Ferlay J, Pisani P Globocan. 2000. Cancer Incidence Mortality and Prevalence Worldwide, Version 1.0. Lyon: IARC Press.
4. Parkin DM, Bary FI, Devesa SS. Cancer Burden in the year 2000. The Global Picture. European J Cancer 2001;37:54-56.
5. Talukder H, et. al. 2005. Annual Report 2005. Bangladesh National Institute of Cancer Research and Hospital, Directorate General of Health Services, Ministry of Health and Family Welfare.
6. Bhatla N. 2001. Jeffocoate's Principles of Gynaecology. 5th Edition. New Delhi: Arnold Company, pp. 390-450.
7. Ponten J, Adami H-O, Bergström R, et al. Strategies for global control of cervical cancer. Int J Cancer 1995; 60:1-26.
8. Zur Hausen H. Papillomavirus acusing cancer: evasion fromhostcell control in early events in carcinogeneis. J Natl Cancer Inst. 2000;92:690-698.
9. Syrjanen K, Syrjanen S. 2000. Papilloma virus infections. In Human Pathology. Chichester: J Wiley & Sons. 1-615.
10. CDCP. 2005. Centers for Disease Control and Prevention. Genital HPV infection fact sheet. Available at: http:/www.cdc.gov/std/hpv/stdfact.hpv.htm. Accessed July 22.
11. IARC. 2005. International Agency for Research on Cancer (IARC). Cervix cancer screening. IARC Handbooks of Cancer Prevention, vol. 10. Lyon, France: IARC Press; 10:1-88.
12. Bosch FX, Manos MM, Munoz N, et. al. Prevalence of human Papilloma virus in cervical cancer: a worldwide perspective. J Natl Cancer Inst. 1995:796-802.
13. Munoz N, Bosch FX, de sanjose S, et. al. Epidemiologic Classification of Human Papillomavirus types Associated with Cervical Cancer. N Engl J Med. 2003;348:518-527.
14. Gray W. 1995. The pathogenesis of cervical neoplasia, In: Diagnostic Cytopathology. 1st edition. Edinburgh: A Churchill Livingstone, pp. 585-690.
15. Murthy NS, Sehgal A, Satyanarayana L, Das DK, et.al., Risk factors related to biological behavior of precancerous lesions of the uterine cervix. Br J Cancer 1990;32:736.
16. Gaffikin L, Lauterbach M, and Blumenthal PD. Performance of visual inspection of acetic acid foe cervical screening: A qualitative summary of evidence to date. Obstet Gynaecol Surv. 2003;58 (8):543-550.
17. Galgano MT, Castle PE, and Stoler MH, et. al. Can HPV-16 genotyping provide a benchmark for cervical biopsy specimen interpretation? An J Clin Pathol. 2008.
18. Koss LG, Melamed. 2006. Koss's Diagnostic Cytology and Its Histopathologic Bases. 5th edition. A Wolters Kluwer Company: pp.293.
19. Doh AS, Nkele NN, Achu P, et. Al. Visual inspection with acetic acid and cytology as screening methods for cervical lesion in Cameroon. Int J Gynaecol Obstet 2005; 89(2):167-173.
20. De Vuyst H, Claeys P, Njiru S, et. al. Comparison of Pap smear, visual inspection with acetic acid, human Papilommavirus DNA-PCR testing and cervicology. Int J Gynaecol Obstet. 2005;89(2):120-126.

21. University of Zimbabwe and JHPIEGO Cervical Cancer Project. Visual Inspection with acetic acid for cervical-cancer screening: test qualities in a primary-care setting. The Lancet. 2003 353: 869-873.
22. Tayyeb R, Khawaja NP, and Malik N. Comparison of Visual Inspection of cervix and Pap smear for cervical cancer screening. J Coll Physicians Surg Pak. 2003;13(4): 201-203.
23. Hussain M, Nasir TA, et. al. Can VIA replace Pap smear as screening tool for cervical neoplasia: evaluation in 200 cases. Bang J Pathol. 2007;22(2), pp.2-9.
24. Israt T. 2006. Study on HPV-DNA Test and Conventional Pap Test for Identification of Cervical Intraepithelial Lesions and Cancer. MMD Thesis, Bangabandhu Sheikh Mujib Medical University.
25. Begum T. 2006. Correlation between pap smear cytology and visual inspection with acetic acid in the diagnosis of cervical lesions. MPhil Thesis, Sylhet MAG Osmani College, Sylhet.
26. Sankaranarayanan R, Wesley R, Somanathan T, Dhakad N, et. al. Visual inspection of the uterine cervix after the application of acetic acid in the detection of cervical carcinoma and its precursors. Cancer 1998; 83: 2150–2156.
27. Shankaranarayanan R, Wesley R, Thara S, et. al. Test characteristics of visual inspection with 4% acetic acid (VIA) and Lugol's iodine (VILI) in cervical cancer screening in Kerala, India. Int J Cancer 2003;106: 404-408.
28. Sankaranarayanan R, Basu P, Wesley RS, et. al. Accuracy of visual screening for cervical neoplasia: results from an IARC multicentre study in India and Africa. Int J Cancer 2004;110: 907–913.
29. Sankaranarayan R,Chatterji R, Shastri SS, et. al. Accuracy of human papillomavirus screening of cervical neoplasia results from a multicenter study in India. Int J Cancer. 2005;116(5); 830-831.
30. Sankaranarayanan R, Esmy PO, Rajkumar R, et. al. Effect of visual screening on cervical cancer incidence and mortality in Tamil Nadu, India: a cluster-randomised trial. Lancet 2007;370: 398–406.
31. Sankaranarayanan R, Nene BM, Shastri SS, et. al. HPV screening for cervical cancer in rural India. N Engl J Med. 2009. 360: 1385–1394.
32. Belinson J, Qiao YL, Pretorius R, et. al. Shanxi Province Cervical Cancer Screening Study: a cross-sectional comparative trial of multiple techniques to detect cervical neoplasia. Gynecol Oncol 2001;83:439–444.
33. Munoz N, Bosch FX, de sanjose S, et. al. Epidemiologic Classification of Human Papillomavirus types Associated with Cervical Cancer. N Engl J Med. 2003;348:518-527.
34. Cronje HS, Parham GP, Cooreman BF, et al. A comparison of four screening methods for cervical] neoplasia in a in a developing country. Am J of Obstet Gynecol. 2003;188:395-400.
35. De Vuyst H, Claeys P, Njiru S, et. al. Comparison of Pap smear, visual inspection with acetic acid, human Papilommavirus DNA-PCR testing and cervicology. Int J Gynaecol Obstet. 2005;89(2):120-126.
36. Denny L, Kuhn L, De Souza M, et. al. Screen- and-treat approaches for cervical cancer prevention in low-resource settings: a randomized controlled trial. JAMA 2005;294:2173–2181.
37. Syeeda S. 2003. Colposcopic Findings in Clinically Unhealthy Cervix: A study in a group of Patients Attending Colposcopy Clinic at BSMMU; BCPS Dissertation, Dhaka.