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Comparative evaluation of smear cytology & hybrid capture II for the diagnosis of cervical cancer

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***Background & objectives*: There is no ideal screening method for cervical cancer in India with the role of human papilloma virus (HPV) detection yet to be established. This study was undertaken to compare the diagnostic accuracy of HPV testing by hybrid capture II (HC-II) with conventional cervical smear cytology for squamous intraepithelial lesions (SIL).**

***Methods*: This prospective study was conducted at New Delhi during 2003-2004 with patients selected from the gynaecology out patients of the All India Institute of Medical Sciences. Initial screening by a questionnaire and per-speculum examination were used to select high-risk patients. Patients, in whom conventional cytology, HC-II test and colposcopy-directed biopsy were done, formed the basis of this study.**

***Results*: Of the 133 patients included in the study, incidence on biopsy of low grade SIL (L-SIL) was 6.77 per cent, high grade SIL (H-SIL) was 8.27 per cent and carcinoma was 3.00 per cent. Sensitivity and specificity of cytology for detection of H-SIL and above lesions was 93.33 and 83.49 per cent while for HC-II it was 93.33 and 90.83 per cent, respectively. HC-II had higher diagnostic accuracy of 91.13 per cent versus 84.68 per cent for cytology. Kappa for HC-II was higher (0.67) than cytology (0.52). Among patients diagnosed to have atypical squamous cells (ASC-US & ASC-H) and L-SIL, HC-II helped to select patients who had significant lesions on biopsy.**

***Interpretation & conclusion*: The main utility of HC-II is in the triage of patients with cytology smear diagnosis of ASC-US, ASC-H or L-SIL, for referral to colposcopic examination. HC-II alone has the best diagnostic accuracy but owing to high cost it is unsuitable for general screening in developing countries. Combining HC-II with cytology will refer smaller numbers for colposcopy, improving efficient utilization of available resources.**

Key words Cervical cancer - cytology - hybrid capture II - screening - squamous intraepithelial lesion

Carcinoma of the uterine cervix is a serious health problem in India[1,2]. Since it has a well-defined precancerous lesion termed squamous intraepithelial lesion (SIL)[3,4] and progression from SIL to carcinoma is slow[5,6], occurring over many years, screening based cervical cancer prevention has been highly successful[6]. Human papilloma virus (HPV) is associated with cancerous as well as precancerous lesions of the cervix[8,9]. The mechanisms of transformation by HPV are partially understood. The role of HPV testing as an adjunct in screening is under investigation. Due to manpower and resource requirements, screening the general population of India with cytology is not practical. As part of a large study examining screening for cervical cancer, this study was undertaken to compare the diagnostic accuracy of HPV testing by hybrid capture II (HC-II) with conventional cervical smear cytology. HC-II, a second-generation test, is a method to detect any of the 13 carcinogenic types of HPV as a group (HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 & 68)[10]. The study population was restricted to high risk individuals for more focused application and more meaningful evaluation of HC-II.

Material & Methods

This was a prospective study conducted at the All India Institute of Medical Sciences, New Delhi. Selective screening was done from amongst patients seen at the out patient department of gynaecology & obstetrics from January 2003 to December 2004. Patients were selected after an initial screening using a questionnaire, followed by per-speculum examination by a gynaecologist. Consecutive patients presenting with one or more of the following: postcoital bleeding, intermenstrual bleeding, severe discharge per-vaginum or unhealthy cervix on per-speculum examination were included, excluding frank carcinoma cases. These patients were considered high risk. All patients underwent routine cervical smear cytology and HC- II test. Colposcopy was done in all patients and a biopsy was performed when a significant lesion was seen on colposcopy. Patients were called again for biopsy when either cytology report or HC-II was positive, if biopsy had not already been performed. This preliminary report is based on 133 consecutive patients who had been subjected to all of three investigational modalities *viz*., conventional cervical smear examination, HC-II test, and colposcopy-directed biopsy.

Cervical smears were made using Ayer's spatula and endocervical brush. Specimen for HC-II was obtained in the same sitting, immediately after making the conventional cytology smear, using a conical brush and a vial containing the specimen transport medium (STM), both obtained from the manufacturer of HC-II (Digene Corporation, Maryland, USA). Samples were kept refrigerated at -80°C until the test was performed.

Conventional cervical Papanicolaou stained smears were examined by two independent observers and classified according to the Bethesda system, 2001[11]. Biopsies were interpreted without prior knowledge of cytology and diagnosed as normal, inflammatory or cervical intraepithelial neoplasia (CIN). CIN was graded as CIN-I, CIN-II and CIN-III following standard criteria[12]. Diagnoses of CIN-II and CIN-III on biopsy were combined to get "high grade SIL (H-SIL) on biopsy" while CIN-I (with or without koilocytosis) was equated to "low grade SIL (L-SIL) on biopsy" for comparing with cytology. Subsequently, discrepant cases were reviewed by a panel of three pathologists on a multihead microscope to arrive at a consensus diagnosis.

HPV testing was done using HC-II assay (Digene Corporation, Maryland, USA) following the manufacturer's instructions. The result of this test was expressed as relative light unit (RLU), which was converted into a ratio of specimen RLU to the positive cut-off sample RLU. A ratio of >1 was taken as cut-off value for positive, following the manufacturer's instructions. Diagnostic accuracies of cytology alone, HPV DNA testing alone and cytology-HPV combined together were determined using final biopsy diagnosis as the gold standard and kappa was calculated.

Results

Histology: A total of 131 patients underwent colposcopy directed biopsy while in two patients endocervical curettage was done. All biopsies without squamous epithelial lining were considered unsatisfactory for the study (4 cases). Of the rest, 105 biopsies were negative for squamous epithelial lesions and 24 biopsies had squamous epithelial lesions of which 9 had L-SIL, 11 had H-SIL and 4 had invasive carcinoma (Table I). Of the four carcinoma cases, 3 were squamous cell carcinoma and 1 was adenocarcinoma. The latter was diagnosed as adenocarcinoma on review, but the biopsy was small and initially reported as atypical glandular fragments-suspicious for carcinoma. A repeat biopsy was requested, however, the patient was lost to follow up.

Cytology: Of the 133 patients, 5 had unsatisfactory smears. Of the 128 satisfactory smears available, 95 were negative for epithelial lesions while the remaining 33 smears showed squamous or glandular cell abnormalities (Table II).

Of the 33 smears showing epithelial abnormality, high grade-SIL (H-SIL) was seen in 16 smears and low grade-SIL (L-SIL) in 5. Atypical squamous cells of undetermined significance (ASC-US) or atypical squamous cells-cannot rule out high grade SIL (ASC-H) were together diagnosed in 10 smears, of which ASC-US was in 7 and ASC-H in 3 smears. Atypical glandular

Table I. Diagnosis of cases based on histology

Broad histology diagnosis (N)	Details of histology diagnosis	Number of cases
Unsatisfactory* (4)		4
Negative** (105)	Normal	6
	Chronic cervicitis with or without metaplasia	84
	Squamous metaplasia only	13
	Squamous epithelial hyperplasia only	2
Squamous epithelial lesions (24)	L-SIL	9
	H-SIL	11
	Squamous cell carcinoma+	3
	Adenocarcinoma	1
Total (133)		133

*Cervical biopsies without squamous epithelial lining
**Cases negative for epithelial lesions
+Of the 3 cases of squamous cell carcinoma, 2 were micro invasive while 1 was widely invasive
L-SIL & H-SIL - low and high grade squamous intraepithelial lesions

Table II. Diagnosis of cases based on cytology

Broad cytology diagnosis (N)	Details of cytology diagnosis	Number of cases
Unsatisfactory* (5)		5
Negative** (95)	Normal	4
	Inflammation (non-specific)	88
	Inflammation (specific)+	3
Squamous epithelial lesions (33)	ASC-US	7
	ASC-H	3
	AGUS	2
	L-SIL	5
	H-SIL	16
Total (133)		133

*Cases with scant, non-representative smear
**Cases negative for epithelial lesions
+Of the 3 cases, 2 had *Trichomonas vaginalis* and 1 had *Candida* infection
ASC-IS, atypical squamous cells of undetermined significance
ASC-H, ? atypical squamous cells-cannot rule out H-SIL
AGUS, atypical glandular cells of undetermined lesions
H-SIL, high grade squemous intraepithelial lesions
L-SIL, low grade squemous intraepithelial lesions

cells of undetermined significance (AGUS) was seen in 2 smears (Table II).

HC-II testing: High-risk HPV infection was seen in 25 patients (18.80%). HC-II picked up 14 (93.33%) of 15 H-SIL and above cases on biopsy, missing only the adenocarcinoma case; 6 (66.67%) of 9 L-SIL cases were HC-II positive while 5 (4.76%) normal patients had HC-II positivity (Table III). HC-II had a sensitivity of 93.33 per cent and specificity of 90.83 per cent for detecting H-SIL and above cases (Table IV). Combining cytology and HC-II did not improve diagnostic accuracy taking overall cases. Kappa value for HC-II (0.67) was higher than cytology alone (0.52) or HC-II combined with cytology (0.43), when any epithelial lesion was taken as positive screening result. When H-SIL and above lesion on cytology was taken as positive, kappa of cytology (0.82) was more than HC-II alone (0.66) or when combined with HC-II (0.63) (Table IV). ASC-US/ASC-H diagnosis on cytology was rendered in 10 patients (7.52%). Of these, one (10%) was H-SIL and one was L-SIL on biopsy. Both cases were positive for high-risk HPV. One out of the remaining eight ASC-US/ASC-H cases also showed HC-II positivity. Using HC-II on this small group of ASC-US/ASC-H patients therefore helped to refer a smaller group of three patients for biopsy with 100 per cent sensitivity for picking up significant lesions. Five smears were diagnosed as L-SIL on cytology. Of these, only one was L-SIL on biopsy and was also HC-II positive. The remaining four cases were negative on biopsy and were also HC-II negative. Thus HC-II helped to select patients for biopsy after ASC-US and L-SIL diagnosis on cytology smears.

Discussion

The present study used selective screening as an approach to target high-risk women, with high yield for L-SIL, H-SIL and carcinoma as compared to the general population. This selective screening method was adopted to increase the yield of positive cases; so that the utility of HC-II could be evaluated without unnecessarily increasing the sample size. The frequency of ASC-US/ASC-H, L-SIL and H-SIL on cytology was high since only cases where biopsy was performed have been included and abnormal cytology was one of the indications for biopsy. Large population based studies have found frequency of ASC-US/ASC-H at 5 per cent, L-SIL 1.97 per cent and H-SIL 0.5 per cent[13,14].

Studies on the utility of cytology in detecting patients with biopsy proven H-SIL have shown a low sensitivity of 59-77 per cent but higher specificity of 90-98 per cent[15-18]. In these studies, cytology was evaluated with either ASC-US and above or L-SIL and above being used as the basis for referral after which H-SIL was detected on biopsy. The present study had high sensitivity at 93.33 per cent and specificity at 83.49 per cent for H-SIL and above cases using any epithelial

Table III. Distribution of cases based on cytology, histology and HC-II diagnoses

Cytology Diagnosis	No. of cases	Biopsy									
		Uns		Neg		L-SIL		H-SIL		Ca	
		Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
Unsatisfactory	5	0	0	1	4	0	0	0	0	0	0
Negative	95	0	3	3	82	3	3	0	0	0	1
ASC-US	7	0	1	0	4	1	0	1	0	0	0
ASC-H	3	0	0	1	2	0	0	0	0	0	0
AGUS	2	0	0	0	2	0	0	0	0	0	0
L-SIL	5	0	0	0	4	1	0	0	0	0	0
H-SIL	16	0	0	0	2	1	0	10	0	3	0
Carcinoma	0	---	---	---	---	---	---	---	---	---	---
Total	133	0	4	5	100	6	3	11	0	3	1

*HC-II results are shown as positive or negative
Abbreviations as in Table II
Uns, unsatisfactory
Ca, carcinoma

Table IV. Test characteristics of cytology and HC-II for diagnosing L-SIL, H-SIL and carcinoma of uterine cervix

	Sensitivity	Specificity	PPV	NPV	Diagnostic accuracy	kappa
Any epithelial lesion on cytology taken as positive screening test, H-SIL on biopsy :						
Cytology alone	93.33	83.49	43.75	98.91	84.68	0.52
HC-II alone	93.33	90.83	58.33	99.00	91.13	0.67
Combined cytology and HC-II	93.33	77.98	36.84	98.84	79.84	0.43
H-SIL and above diagnosis on cytology taken as positive, H-SIL on biopsy :						
Cytology alone	86.67	97.25	81.25	98.15	95.97	0.82
HC-II alone	93.33	90.83	58.33	99.00	91.13	0.66
Combined cytology and HC-II	93.33	88.99	53.85	98.99	89.52	0.63

Values are percentages
PPV, positive predictive value
NPV, negative predictive value

lesion on cytology as positive for cytology and H-SIL on biopsy. This higher sensitivity and specificity for cytology may be explained on the basis of high index of suspision in a high risk setting and all cytology smears being screened by consultant pathologists.

HC-II has been studied as a modality for screening of carcinoma cervix and has been shown to have high sensitivity but low specificity, reported as 100 and 85.2 per cent, 95 and 97.7 per cent, 74.8 and 93.4 per cent and 100 and 64.8 per cent respectively for H-SIL and above cases[17,19-21]. In the present study, HC-II has a sensitivity and specificity of 93.33 and 90.83 per cent respectively for detecting H-SIL and above cases. Diagnostic accuracy and kappa for HC-II were higher than for cytology, indicating that it is a better screening test. This has been the experience of numerous recent papers from the west as well as from India[22-27]. In contrast to cervical cytology, which is highly subjective, HPV DNA testing by HC-II is well standardized and large number of samples can be processed together. In addition, results of HPV testing would not be adversely influenced by cervical inflammation and cervicovaginitis, which are common conditions in women in low-resource settings. HPV testing identifies not only women who currently have high grade cervical disease but also women with L-SIL caused by high-risk HPV who are therefore at risk for developing H-SIL and carcinoma in the future. The high negative predictive value of HC-II testing implies that HC-II is the best primary screening test. Even in the setting of screening by consultant pathologists HC-II showed better performance indicators in the present study. However, it is achieved with very high percentage of referrals for biopsy. Large studies have found 16.7 per cent of all screened women to be HC-II positive[19]. Hence HC-II alone cannot be used as a primary screening modality in developing countries in view of its high cost. The total cost of the test is around one thousand Rupees per test, not including manpower cost. Compared to this, cytology smears have a screening cost of three hundred Rupees, excluding manpower cost, but including laboratory setup costs[28]. The manpower cost of HC-II and cytology screening are similar, while consumable and equipment cost per test are three times higher for HC-II compared to cytology[28]. Recently, in a large collaborative prospective study done on south Indian rural women, Franceschi *et al*[23] examined 1943 women for cytology and HPV testing by PCR. Of these, the prevalence of HPV of any type was 16.9 per cent. It was 14.0 per cent among cytologically normal women and 73.9 per cent among those with cytological abnormalities[23]. Similar high prevalence of HPV infection has been seen in Western population as well[22], which implies that HC-II will refer a high percentage of the population for biopsy and colposcopy. This is reflected in the low positive predictive value (PPV) of 58.33 per cent in our study, which was among high-risk patients. However, it is still better than cytology which had a PPV of 43.75 per cent in the present study. Colposcopy with biopsy is estimated to cost around one thousand six hundred Rupees while treatment for significant lesions varies from four thousand five hundred (laser) to thirteen thousand five hundred Rupees (hysterectomy)[28].

The main cervical cancer screening modality for India is still unclear. Screening of general population with cytology is difficult. However, selective cytology screening for high-risk patients used in the present study is highly successful in increasing the yield of abnormal smears and should form a part of the screening strategies, which can be developed for the future. The main role of HC-II is in triage of patients having abnormal cytology smears since high cost prevents utility as a general screening technique. More importantly, colposcopy and biopsy require highly specialized and trained manpower which are a scarce resource in India. HC-II on the other hand, is mechanical and easily automated in large numbers. All patients with diagnosis of ASC-US, ASC-H or L-SIL should undergo HC-II testing with only HC-II positive patients being referred for biopsy. This effectively screens out L-SIL caused by low-risk HPV infection and ASC-US patients for whom biopsy is not needed. It is important to note that L-SIL caused by low risk HPV infection do not cause progression to cancer and do not require treatment as established by the ALTS trial[13]. HC-II will be effective in identifying such L-SIL patients who only need follow up, and the effective utilization of colposcopy services would be achieved.

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