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Evaluating the role of HPV as a primary screening method for cancer cervix: A pilot study

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Abstract
Cervical cancer is the leading genital malignancy in India, there is a dire need to schedule screening programs on a regular basis.
Objectives: 1) To compare the efficacy of visual, cytological and HPV DNA testing as primary cancer screening methods for the detection cervical intraepithelial neoplasia and cancer.
2) Colposcopic evaluation and directed biopsy for positive cases.
Methodology: Prospective observational study including 100 married women in the age group 35-50 years for opportunistic screening were enrolled and they underwent a visual inspection of the cervix followed by cytology (PAP smear) and HPV DNA testing at the same visit.
Results: Visual inspection and Pap smear as screening methods had good specificity but low sensitivity values, as compared to HPV.
Conclusion: HPV testing serves as a more reliable and cost effective screening method, can be considered as a gold standard one step screening method for preinvasive lesions of the cervix.

Keywords: cervical screening, HPV testing

Introduction
Cervical cancer presents a significant global health burden mostly in the developing countries, comprising 12% of all female cancers, approximately 85% of cervical cancers occur in the developing countries [1].
Low socioeconomic status, poor menstrual and reproductive hygiene, lack of population based screening programs have led to high incidence and death rates due to cervical cancer. Cancer screening aims to detect and treat premalignant, low or high grade disease.
Human papilloma virus (HPV) is the causative agent for cervical, vaginal, vulval, anal, penile and oropharyngeal cancers. HPV is a double stranded DNA virus that infects squamous epithelia, including the skin and mucosae of the upper respiratory and anogenital tracts. More than 120 subtypes of HPV have been identified and the viral genome has a common structure with three main areas: E (early), L (late) and the genome regulatory region [1]. Oncogenic HPV types account for 99.7% of cases, common subtypes are 16, 18, 31, 33, 45.
Genital HPV infection acquired through mainly sexual intercourse has a lifetime risk of upto 80% in exposed individuals [1]. Prevalence of HPV infection declines with age but increases with multiple sexual partners. Most HPV infections are asymptomatic and self limiting while 10-15% of persistent infections tend to cause cancers [2].
HPV subtypes 16 and 18 have been found to be the most pathogenic of the high risk types, accounting for 70-80% of squamous cell carcinomas and > 90% of adenocarcinoma of the cervix, 40-50% of vulval cancers and 70-80% of anal cancers [1]. Compared to the risk in uninfected women the risk of developing squamous cell carcinoma of the cervix is about 400 times higher following infection with HPV 16 and about 250 times higher following infection with HPV 18 [3, 4].
The most common symptoms of cervical cancer include intermenstrual, postcoital or postmenopausal bleeding, rarely with vaginal discharge, dyspareunia or pelvic pain.
Cervical cancer has a predictable disease progression period and so there is scope for cancer screening in a view to detect premalignant lesions. Dyskaryosis is the term used to describe an abnormal cytology. Bethesda system of classification is used to classify cervical cytological abnormalities. “Low grade” (CIN 1-2) lesions reflect a transient productive HPV infection

whereas "high grade" (CIN 3) suggest a premalignant lesion with the presence of transforming high risk HPV infection. Borderline changes in the nucleus are termed as atypical squamous cells of uncertain significance.

Cervix is amenable to screening by a number of methods which include visual inspection with acetic acid (VIA), magnified VIA (VIAM) visual inspection with Lugol's iodine (VILI), the Papanicolaou test, and HPV DNA testing.

Primary screening with exfoliative cytology with PAP smear in 1940's then in 2000 liquid based cytology was introduced to improve results. Secondary screening with colposcopy is used to determine appropriate management for women referred with an abnormal cytology result. A guided biopsy may be taken from the cervix for confirmatory diagnosis. Cervical intraepithelial lesion (CIN) is a histological term that describes squamous premalignant change.

HPV has a stable double stranded DNA genome that can be obtained from exfoliated cells, detected by DNA based assays. HPV testing is a simple cost effective, feasible and acceptable screening tool with a high sensitivity for detecting cervical intraepithelial lesion (CIN) 2/3 or early invasive cancer, as compared to cytology and visual inspection. In borderline cases on cytology, HPV testing helps in segregating negative and positive women and then further follow up [5, 6].

The lifetime risk of an infection with high risk HPV is high; however, only a minority of infections develop into cervical cancer. After 12 months, two-thirds of all infections can be cleared by the host immune system, and after 24 months, over 90% can be cleared.

The risk factors associated with HPV are lower age, lower educational level, non-white ethnicity, current and previous oral contraceptive use. In many cases, HPV causes no symptoms. When they do occur, the most common symptom is warts in the genital area. Signs of infection can appear weeks, months, or even years after the person has been infected with the virus.

In HPV triage, PAP smear with high grade dyskaryosis need colposcopy and biopsy, with a borderline or low grade dyskaryosis need HPV testing and then colposcopy or biopsy if needed. HPV testing can also help in cases with follow up of cases following conservative therapies.

However its inability to distinguish transient infections and clinically relevant infections results in limited specificity compared to cytology. Referrals for colposcopy could rise.

Studies have demonstrated a sensitivity of 96.1% and specificity of 90.7% for HPV versus cytology which has a sensitivity of 53% and specificity of 96.3% [1].

Its high sensitivity can significantly improve the effectiveness of screening programs and its prolonged negative predictive value can allow extension of screening intervals. A negative HPV will be satisfying for the patient as next schedule would be after 6 years or more, while a positive test is stressful for the patient to understand the consequences of the infection and further follow up is required.

HPV testing as a primary screening method for cervical screening has shown to be effective with 60-70% protection against cervical carcinoma as compared with cytology and visual inspection [1, 6].

More so with cervical glandular intraepithelial neoplasia where HPV 18 and 45 are commonly associated and skip lesions are common and most often conservative therapies may not confer benefit.

Given the heavy disease burden of cervical cancer, prophylactic HPV vaccines were developed to target the commonest high- and low-risk HPV genotypes. HPV vaccination with Gardasil (HPV 16/18/6/11) and Cervarix (HPV 16/18) with two doses make it more acceptable and cost effective in reducing the incidence and morbidity associated with HPV infection [2].

Objectives

1. To compare the efficacy of visual, cytological and HPV DNA testing as primary cancer screening methods for the detection of premalignant lesions and invasive cervical cancer,
2. Colposcopic evaluation and directed biopsy for positive cases.

Materials and Methods

Prospective observational study including 100 married women in the age group 35- 50 years attending the gynaecological outpatient department for various gynaecological complaints were counselled for opportunistic screening and enrolled in the study. Married women willing to participate in the study and fulfilling the inclusion criteria were taken into account, after a brief interview about patient demographics, informed consent was taken and then women underwent a visual inspection of the cervix followed by cytology (PAP smear) and HPV DNA testing at the same visit.

During a speculum examination, trained gynecologists visualized the cervix for abnormalities like unhealthy looking cervix, infections, discharge, bleeds on touch, growth on the cervix and then collected ecto- and endo-cervical cells for Pap smear.

After removing any obscuring mucus from the cervix with a cotton swab, exfoliated ecto and endocervical cells were collected and smeared onto a glass slide using an Ayres spatula and fixed by placing the slides in ethanol. Slides being labelled and sent to the pathology laboratory for reporting under Bethesda classification.

For HPV DNA testing a sterile cotton swab was used to rotate it in the cervical canal and the swab was transferred immediately to the microbiology lab for polymerase chain reaction testing and interpretation.

On follow up, patients with inflammatory smears were treated and HPV negative were reassured. HPV positive ones were counselled for colposcopy and biopsy and follow up advised.

For the women randomized to receive colposcopy at enrollment, the colposcopic examination was performed after collection of specimens for Pap smear and HPV DNA testing and after conducting VIA.

PAP reported according to Bethesda system, which could be normal, inflammatory, low grade or high grade precancerous lesions or invasive cancer.

HPV positive status will again depend on the subtypes like high risk types, viral load, age parameters and risk factors on the patient's side.

Women with positive HPV testing results were scheduled for a colposcopy with or without biopsy. Conservative procedures like cryotherapy, large loop excision, cone biopsy for CIN I –II may be needed after histopathological reports. If colposcopy is normal or has features of chronic cervicitis she can be scheduled for follow up with cytology after 12 – 24 months in which time 90 % of HPV infections will be self cleared.

PAP and HPV negative, will be advised for follow up with cytology once in 6 – 10 years. People with PAP positive and HPV negative can be reassured and follow up with PAP after 3 - 5 years, PAP positive and HPV positive or PAP negative with HPV positive will be called for colposcopy with or without biopsy and if negative, then follow up with cytology once in 5 – 6 years.

Sensitivity, specificity, positive and negative predictive values were analyzed statistically.

Results

Total study group: 100

Table 1: Visual inspection

1.	Normal	84
2.	Suspicious	4
3.	Abnormal	12

Table 2: PAP results

1.	Normal	55
2.	Inflammatory	38
3.	LSIL	5
4.	HSIL	1
5.	Invasive	1

Table 3: HPV Results

1.	Positive	48
2.	Negative	52

Table 4: Colposcopy results - total of 48 cases

1.	Normal	40
2.	Suspicious	2
3.	CIN 1changes	8

Table 5: Biopsy results – Total of 48 cases

1.	Normal	16
2.	Infection – chronic cervicitis	20
3.	Precancerous	8
4.	Malignant	2

Table 6: Statistical analysis of Visual inspection and PAP smear results.

	Sensitivity	**Specificity**	PPV	NPV
Visual Inspection	33.33% (20.40%-48.41%)	100% (93.15%-100.0%)	100% (93.15%-100.0%)	61.90% (57.09%-66.50%)
PAP	66.67% (51.99%-79.67%)	(75.00% (61.05%-85.97%)	71.11% (59.61%-80.41%)	70.91% (61.33%-78.93%)

Table 7: Comparison between Visual Inspection and HPV cases.

Visual Inspection	**HPV**					
	Positive (n)	**%**	**Negative (n)**	**%**	**Total (n)**	**%**
Normal	32	66.7	52	100.0	84	84.0
Suspicious	4	8.3	0	0.0	4	4.0
Abnormal	12	25.0	0	0.0	12	12.0
Total	48	100.0	52	100.0	100	100.0

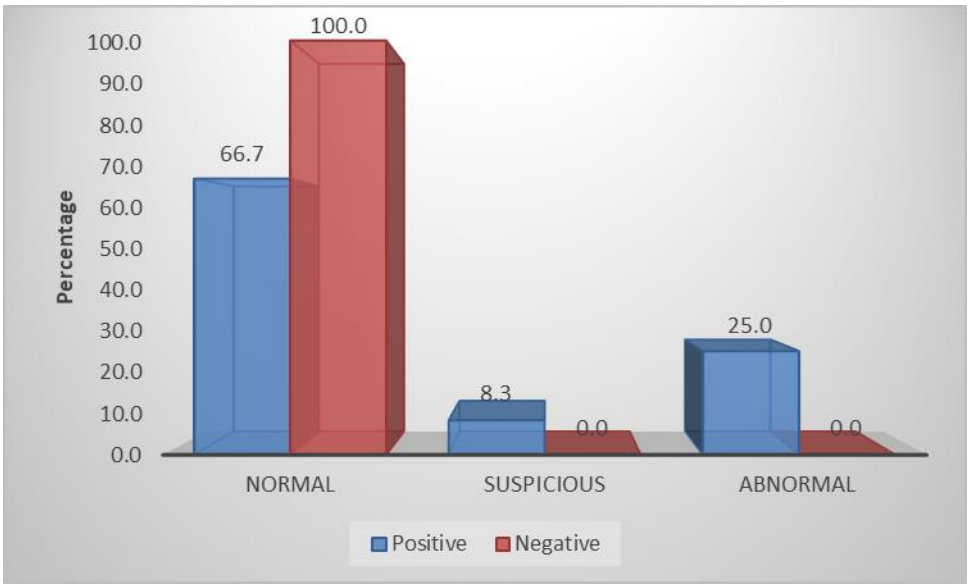

Fig 1: Visual Inspection

Table 8: Comparison between PAP and HPV

PAP	**HPV**					
	Positive	**%**	**Negative**	**%**	**Total**	**%**
Normal	16	33.3	39	75.0	55	55.0
Inflammation	20	41.7	13	25.0	33	33.0
ASCUS	5	10.4	0	0.0	5	5.0
LSIL	5	10.4	0	0.0	5	5.0
HSIL	1	2.1	0	0.0	1	1.0
Invasive	1	2.1	0	0.0	1	1.0
Total	48	100.0	52	100.0	100	100.0

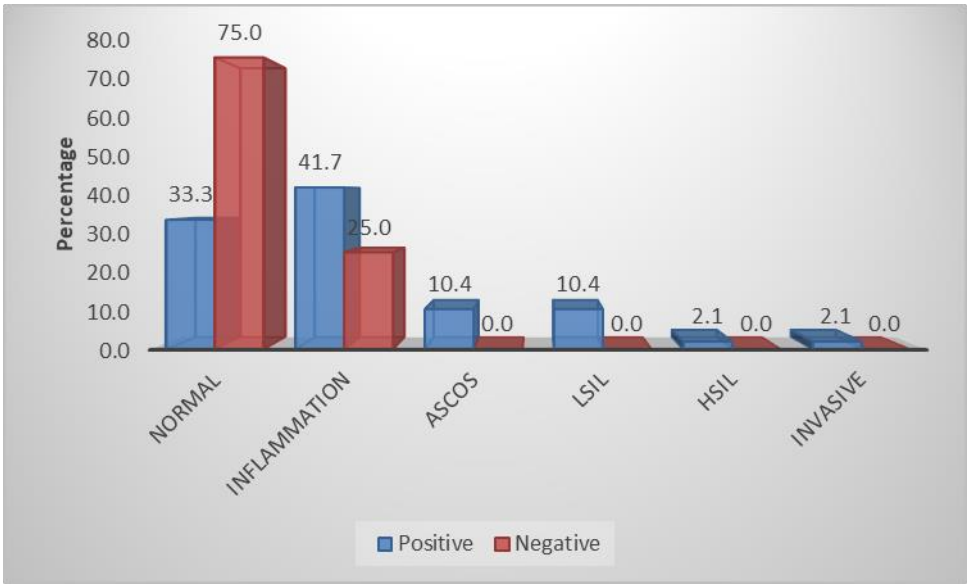

Fig 2: PAP

Table 9: Follow up of HPV positive cases

HPV Positive Cases	Colposcopy +ve	Colposcopy -ve	Biopsy +ve	Biopsy –ve
48	10 (20.8%)	38 (79.12%)	30 (62.5%)	18 (37.5%)

Visual inspection

Among the study population, visual inspection for detection of preinvasive lesions of the cervix had 33% sensitivity with 100% specificity, a positive predictive value of 100%and negative predictive value of 61.90%.

Pap smear

Pap smear showed a sensitivity of 66% and specificity of 75%, a positive predictive value of 71.11%and a negative predictive value of 70.91%.

Among the 48 HPV positive cases, all were subjected to colposcopy and guided biopsy, of which 20.8% had positive preinvasive cervical findings on colposcopy and 62.5% of them did show proved preinvasive lesions on histopathological examination.

Visual inspection and Pap smear as screening methods had good specificity but low sensitivity values, as compared to HPV, which may need further testing for confirmation.

HPV testing serves as a more reliable and cost effective screening method, can be considered as a gold standard one step screening method for preinvasive lesions of the cervix. This will reduce the burden of further ongoing screening schedule.

Discussion

Screening for cervical cancer using HPV testing is more sensitive than screening using cytology but considerably less specific. The advantages of high risk HPV testing over cytology (Currently the most widely used screening method) are that HPV negative women are extremely unlikely to develop cervical cancer over the next 5-10 years and infrequent screening would be safe. Additionally HPV testing does not depend on collection of samples or adequacy of cellular morphology.

VIA is not recommended for women over age 50 years. We did not place an upper age restriction for participation in this study because we felt that in our population of women who had never been screened, everyone could benefit from any of the applied screening tests, however HPV testing performance is not compromised in older women.

HPV DNA testing was both more sensitive and specific than Pap cytology and VIA. The use of a less invasive and more user-friendly primary screening strategy (Such as swabs for HPV DNA testing) may be required to achieve the coverage necessary for effective reduction in cervical cancer mortality [7].

HPV DNA testing of women 30 years and older, at least once in their lifetime can result in a 36% reduced lifetime risk of cervical cancer, so cost effective. Primary HPV testing allows earlier identification of women who need treatment and needs to be implemented in population based cervical screening.

Studies have proven that colposcopy rates are not increased following HPV testing rather it benefits that in the correct age group, the screening intervals are increased from 3 yearly to 5-6 yearly or more. The drawback is that there is a need for international standardization and quality assurance with optimization and evaluation of the method in real life setting [8].

HPV testing as a screen for cervical cancer has been tested in various randomized controlled trials and it can predict the risk of future development of cervical cancer [9].

Newer strategies in cervical screening are the use of samples for HPV testing reported to be more cheap and effective than cytology-based screening, with a sensitivity/specificity between 60 to 90% [10]. A more scheduled implementation of the program is needed to address the following 1) the screening groups and means of contact, 2) the primary testing and triage approach, 3) rendering of accurate final diagnoses, 4) treatment, follow-up and documentation [10].

Primary high risk HPV screening is more scientific and clinically advanced screening tool for cervical screening since it offers better reassurance of low cancer risk compared to cytology-only screening conducted at the same interval [11].

Limitations of the study

Small study population, needs to be implemented on a larger scale. Probably the study population belongs to high risk group.

Conclusion

HPV DNA testing was both more sensitive and specific than Pap

cytology and VIA HPV testing appears to be the preferred approach towards primary cervical cancer screening in both high and low resource setting. Testing of HPV in cervical samples offers a relatively cheap and effective way of improving screening coverage.

The time has come to implement, exploit the potential of high risk HPV testing as a primary screening test in population based cervical screening for improved cost efficiency in detecting precancerous lesions of the cervix when compared to cervical cytology.

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