Human papillomavirus infection (HPV) & screening strategies for cervical cancer

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The incidence of cervical cancer is declining slowly necessitating concerted and organized control measures. Control through primary prevention has become a distinct reality though a prophylactic vaccine, which may take quite some time for its widespread use. Thus control of cervical cancer through secondary preventive measures is the only viable solution now. While high quality cytology screening may not be feasible for widescale implementation in developing countries because of lack of necessary infrastructure, quality control and poor sensitivity of cytology, alternative screening modalities such as visual screening techniques and HPV-DNA can be explored. Some technical and feasibility aspects of these three modalities are discussed.

Key words Cervical cancer - HPV - cytology-based screening - HPV-DNA - visual screening

Human papillomavirus infection (HPV) causes about 466,000 new cases and 231,000 deaths due to cervical cancer annually: 80 per cent from developing countries. A World Bank report estimated that women with cervical cancer die about 18 years earlier than they would have otherwise. In India, an estimated 1.5 lakh women develop cervical cancer annually, about 16 per cent of the world annual incidence. Thus, cervical cancer is an important public health problem that deserves urgent attention.

For the control of cervical cancer, there are two approaches: primary and secondary prevention. Primary prevention involves a risk-reduction approach through behavioural intervention for sexual and health care seeking behavior or through the mass immunization against high risk HPVs. Secondary prevention involves screening for precancerous lesions and treating them. The three screening modalities are cytology, visual inspection, and HPV detection.

Screening modalities: Natural history models

A clear understanding of the natural history of cervical cancer is a key to planning and implementing a rationale screening programme. Since cervical cancer is caused by infection with a range of high risk “oncogenic” HPV types, the natural history of cervical cancer is essentially that of HPV infection.

Accepted models of cervical cancer natural history have changed in recent years. Earlier the cervical cancer prevention programs were based on the premise that disease developed from precursor lesions, progressing steadily from mild to moderate to severe dysplasias to carcinoma in situ (CIS) and then to cancer. The current cervical carcinogenesis model includes 3 steps of HPV infection, progression to high grade preinvasive lesions and invasion. Most infections (>95%) including those with cytologic
abnormalities, resolve spontaneously, returning to HPV DNA negativity, often with seropositivity5,6. The direct precursor to cervical cancer is high grade dysplasias (HSIL) which can progress to cervical cancer over a period of up to 10 years.

When the virus infects a mitotically active epithelial cell, it may result in a latent infection. The cells with latent infection appear morphologically normal, and thus cannot be detected by cytology or through visual techniques of examining cervix. At this stage the only screening modality of HPV detection through molecular techniques is feasible7. Once the latent infection enters into a replicative state, the viral replication takes place irrespective of the cell cycle. The cytologic and histologic changes associated with productive HPV infection are generally referred to as “low grade squamous intraepithelial lesion” (LSIL). Colposcopically, replicative HPV infection manifest as acuminate warts or flat warts in the cervix or elsewhere in the genital tract8. Thus, cytology or visual strategies for screening become operative only at this stage of productive stage of HPV.

The 3rd possibility is the neoplastic transformation with or without passing through a productive phase. In this stage the virus clearance doesn’t take place and the infection tends to persist. This usually happens with the virus belonging to high or intermediate risk groups. Progression to high grade squamous intraepithelial lesions (HSIL) or invasive cancers is rather uncommon, occurring in only 3-10 per cent of infections. It appears that these changes are usually preceded by integration of the virus into the host chromosomal DNA9.

Most LSILs regress especially, incidental cases in younger women below 35 yr of age. Prevalent cases are less likely to regress. Thus, the natural history suggests that the screening initially should focus on women at the highest risk of precancerous lesions – women in their 30s and 40s. Cervical cancer most often develops in women after age 40 and peaks around age 50. Precursor lesions remains detectable for up to 10 years before cancer develops, with a peak SIL rate at about 35 years. Natural history model and clinical data suggest that cervical cancer generally develops slowly from precursors. Therefore, screening can take place infrequently and still have a significant impact on morbidity and mortality. Screening every 3 years has almost as great an impact (91%) as screening every year (93% reduction in incidence). Even screening every 10 years can have a significant impact (64%) on incidence10. Screening emphasis, then, should be on coverage rather on frequency.

**Cytology-based screening**

The procedure involves scraping cells from the squamocolumnar junction of the cervix and then fixing them on a glass slide (pap smear). The slides are sent to a cytology laboratory and evaluated by a trained cytologist or cytotechnician who determines the cell classification as atypical squamous cells of undetermined significance (ASCUS), low grade squamous intraepithelial lesions (LSIL) and high grade squamous intraepithelial lesions (HSIL).

A strong correlation is observed between initiation of cytology screening and reduction in incidence and mortality from cervical cancer. Efficacy of cytology based screening programmes have been adequately demonstrated in countries such as Denmark, Finland, Iceland, Norway, Sweden and British Columbia11-13. Cytology has limitations as high rate of false negative results due to vagaries of lesion distribution in the cervix, exfoliative variability in cell samples, smear preparation and staining, microscopic reading, and variations in the reader’s stringency in interpretation and reporting13. Several meta-analyses have reported quite low pap smear sensitivities - with a median of 50 per cent13-15. The sensitivity falls further for post menopausal women due to physiological changes of the cervix. In general, the low sensitivity of a single pap test makes it necessary to screen women relatively frequently - every 3 to 5 years, a proposition not suitable for developing countries where even once a life time screen is not feasible.

Many developing countries that have initiated cytology based programs have yet to witness a reduction in cervical cancer burden. For instance, Mexico, where national Pap smear screening programme has been in place since 1974, the mortality rate remained steady for 15 years at 16 per 100,00016. Recently in a clustered randomized trial in Osmanabad district of India, one round of cytoscreening in women aged 30-59 yr failed to show any reduction of mortality or number of advanced cervical cancer after eight years17.

A meta analysis of 62 studies showed14 the mean specificity to be 68 per cent only (range 14-97%). Though broad range of specificity was obtained the pap smear is considered to be specific with regard to detection of high grade lesions and cancer. This aspect has significant clinical implications when referrals are
made for HSIL and higher lesions only as it obviates the need for unnecessary biopsy.

For conventional pap smear, the relative proportion of sampling to screening errors are about 2:1 that can be reduced using several new technologies to improve the accuracy. While these approaches appear promising, they are expensive and rely heavily on technology\textsuperscript{18}. Fluid-based, thin layer processing of cervical samples attempts to reduce sampling errors and improve specimen adequacy by suspending cervical cells in a liquid solution. This method makes it easier to successfully evaluate cervical cells. The other technology is Automated pap testing, which attempts to reduce laboratory interpretation errors by using computerized analysis to evaluate pap smears. There is a growing body of evidence that liquid based cytology addresses the limitations of smear methods and could be a cost effective alternative to conventional smear cytology in developed countries\textsuperscript{19}.

Pap smear screening efforts can succeed only when implemented in an environment that has reliable infrastructure\textsuperscript{18}. Minimum requirements for establishing an effective pap smear screening include well trained pap smear providers, initial and ongoing access to supplies and equipments, linkages including transportation, to a reliable cytology laboratory, proven system for timely communication of test results to screened women, and effective referral system for diagnosis and treatment. When any of these key requirements is missing, cytology programs are not likely to be successful. Cytology based screening is resource-intensive, labour-intensive and is highly subjective. Even adoption of newer cytology techniques does not change its subjective character.

**Visual approaches to screening**

It involves 3 different approaches: Visual inspection of cervix with acetic acid (VIA), visual inspection with magnification (VIAM), and visual inspection after application of Lugol’s iodine (VILI).

**VIA:** It involves swabbing the cervix with 3-5 per cent acetic acid (Vinegar). After 30-90 seconds, a transient reaction occurs due to osmotic dehydration of dysplastic cells, which accentuate the optically dense chromatin to aceto white areas. VIA is a simple and inexpensive low technology test that does not require a laboratory infrastructure and providers can be trained in 1 to 2 wk. Consumables required are cheap and universally available. The test results are available immediately, enabling further investigation/treatment to be performed in the same session. This avoids recall of women for procedure, resulting in logistic advantages, better compliance and cost savings\textsuperscript{20}.

The sensitivity of VIA for high grade lesions and invasive cancer ranged from 70.9-82.6 per cent and specificity from 64.1 to 86.5 per cent in cross sectional studies in Zimbabwe, China and India\textsuperscript{21-23}. VIA has a higher sensitivity but lower specificity than that of cytology\textsuperscript{24}. Lower specificity of VIA implies a higher number of women requiring additional investigation and treatment. It remains to be seen whether specificity can be improved by further developments in test definitions and training strategies. To date, there is no universally accepted uniform definition of the test results of VIA, and given the considerable variation in the way these tests are applied and interpreted in different settings, standard definitions and approaches are urgently needed.

Lesions viewed by VIA vary in their size, whiteness, opacity and margins. Unlike cytology, where different grades of severity are stated as ASCUS, LSIL, HSIL, invasive cancer, VIA is recorded as positive, negative, inadequate or doubtful. Acetowhitenning may be due to many causes other than dysplastic epithelium. To implement appropriate medical protocols, health care providers must carefully consider the features of a lesions. Thus feasibility of VIA based screening is dependent on training and monitoring\textsuperscript{24}.

Concerns have been expressed about reproducibility and quality control of VIA in field conditions\textsuperscript{25}. It has been shown in the Barshi trial that the test positivity of VIA declined from 17 per cent at the beginning of the project to 10 per cent by the middle of the project, after a brief period of retraining.

A VIA based screening programme may be more readily integrated into primary care health service in developing countries. Model based simulations of cost-effectiveness indicate that cervical cancer screening strategies that incorporate VIA and eliminate colposcopy may be attractive alternatives to cytology\textsuperscript{25}.

While in a cluster randomized trial in Dindigual involving over 49,000 women VIA intervention showed reduction in both incidence and mortality by 25 and 35 per cent respectively in 7 years after one round of screening\textsuperscript{26}, a similar trial in Barshi, Osmanabad district, failed to show any reduction in mortality\textsuperscript{17}. The reasons for these discordant results are not known, however.
**Visual inspection with magnification (VIAM):** VIAM is the visualization of cervix under low magnification after application of acetic acid. It is not yet known whether use of magnification offer a significant advantage over VIA. Investigation by Basu *et al* \(^{27}\) and Sankar *et al* \(^{24}\) showed that VIAM was not superior to VIA, even there was some loss of specificity. Magnification did not give any improvement in detection rate of high-grade dysplasia or cancers over the use of VIA in studies from South Africa \(^{28}\), and from Kolkatta \(^{27}\).

**Visual inspection after application of Lugol’s (VILI):** VILI is the visualization of cervix after application of Lugol’s iodine. VILI is considered positive, if yellow iodine non uptake areas are visualized close to the squamo columnar junction or if the entire cervix or a growth on the cervix turned yellow. Among the visual test assessed, VILI seems to be particularly promising, detecting 75 per cent of all cases of HSIL compared with VIA and VIAM which detected less than two third of cases. The pooled sensitivity of VILI 91.8 per cent (range 76-97.3%) has been shown to be higher compared to those of VIA (76.9%) and VIAM (64.2%) \(^{28,29}\). The other advantage cited for VILI is that the yellow colour changes associated with a positive VILI test result could be recognized with much greater ease by trained health workers compared with the aceto white lesions associated with VIA. The major disadvantage of VILI is the low specificity of the test. One of the difficulties with VILI is that staining effects persist for 30-45 min and diagnostic procedure such as colposcopy after VILI must be delayed for this period of time, if it is to be administered on the same day.

Advantage of visual techniques has been very high negative protective value more than 99 per cent. A woman negative by VIA/VILI, need not further undergo any investigation. These women may, however be advised to undergo a VIA or VILI after a minimum interval of 3 years. Only 10-15 per cent women who are test positive with visual techniques require further evaluation. These characteristics make them ideal alternative tools for primary screening.

**HPV screening**

Worldwide interest has grown in the potential for HPV testing in cervical cancer prevention programmes, both as an adjunct to cytological screening approaches and in primary screening. Its justification stems from 2 consistent observations. First HPV testing has an average 25 per cent greater sensitivity but about 10 per cent lower specificity than cytology for detecting HSIL \(^{20,30}\). Second the combination of cytology and HPV testing attains sensitivity and negative predictive values that approach 100 per cent \(^{20,30}\). Therefore, use of combination of pap cytology and HPV in a screening programme could potentially allow increasing testing intervals safely e.g., from 1-3 years to 3-5 years or even longer.

The WHO Eurogin joint expert’s conference 2000 (Paris) brought out following consensus statement regarding primary screening with HPV\(^{20}\). HPV testing is objective and highly reproducible in sharpp contrast to conventional as well as liquid based cytology which are subjective and labor intensive. Primary HPV infection and minor cytologic and histologic lesions with little risk of progression to cervical cancer are very common in women under the age of 30, and cervical cancer is rare before this age. In consideration of these facts and harmful consequences that occur from over screening and over treatment of young women, the ideal age to begin screening is approximately 30 years. The very high sensitivity (88 to 100%) of HPV testing for high grade cancer precursors and the declining level of HPV detection in normal women over the age of 30 establishes testing for HPV as a more effective primary screen for women over this age than currently practiced cervical cytology. The extremely high NPV (99-100%) of the combination of normal cytology screen and a negative HPV test should allow safely lengthening intervals up to 8-10 years. The specificity of HPV for HSIL and above ranges from 57-89 per cent. Though the specificity of the test increases in older women, the overall specificity is low. This indicates potential for overt treatment, although some studies currently are exploring the use of a higher cut off value for positivity to address this problem. Testing for persistent HPV infection should take into account the usual clearance time reported for transient HPV infections (7-8 month) when designing management protocols for women with HPV positive high-risk HPV tests.

**Assay system**

With increased standardization of HPV DNA testing methods in 1990’s, reliable data now have emerged from large scale screening programmes. Polymerase chain reaction (PCR) and Hybrid Capture HPV DNA assay (HC II) from Digene diagnostics have become the most frequently used tests for screening purposes. Reliable, sensitive HPV testing methods,
such as MY09/MY11 consensus primer PCR and GP5+/GP6+ general primer PCR which type the wide range of genital HPVs have been well standardized. PCR system had an analytical sensitivity of 10-100 copies of HPV-DNA per sample.

The modified hybrid capture system (HC II) uses 13 probes for high risk HPV types (16,18,31,33,35,39,45, 51,52,56,58,59 and 68). The chosen analytic sensitivity limit of the HCII assay for high risk HPV types was 1 pg/ml (corresponding to 5000 or more HPV DNA copies). Thus, this assay systems is quantitative and cut-off values can be varied to change the sensitivity and specificity of the system.

From a logistic point of view, however, the HCI system is a simpler and easier test to perform. Although the HCI system was not as sensitive as HCII system, at specificities (i.e., prevalence of HPV in general population) likely to be practical for a primary screening test in a low resource setting, HCI assay system performed equivalently to the HC II system. In the context of screening, good sensitivity has to be balanced against the test’s specificity. One of the advantages of HPV-DNA testing in these setting is that the specificity of the test can be altered by adjustment of the cut off levels used to define a positive result.

An excellent overall agreement has been seen between the performance of HCII assays and MY09/ MYO11 RLB systems (agreement 94.8%, kappa 0.89). In another study, the sensitivity of PCR and HCII was 88.2 per cent (78.9-93.8) and 90.8 (83.1-95.8) respectively and the specificity 78.8 (77.9-79.7) and 72.6 (69.4-75).

Major obstacles in conducting HPV screening

1. In developing countries, the key barriers to further exploration of HPV test protocols in low resource settings are cost and technical requirements. In the US, the current HC-II test retails for about US$22 per test, takes 6-7 h to process, and requires access to laboratory equipment and a computer. Thus there is an urgent need to develop HPV tests that are less expensive, rapid and easier to use in the field (non laboratory settings). PATH has launched a “START” project to develop rapid, low cost and easy to use tests to screen for cervical cancer. One of them is a “Batch test” in collaboration with Digene laboratory that will allow processing of multiple specimens in two hours or less, making it suitable for broad-scale campaigns. Another is a “strip-test” in collaboration with Arbor Vita which detects the viral protein biomarker E6, believed to be associated with aggressive precancerous cervical lesions. A cross sectional study in rural China demonstrated that the test characteristics of new developed test kit (Care HPV: QIAGEN, Gaithersburg, MD, USA) are comparable with that of HC-II and that the test could be used as a primary screening test. The CareHPV assay has been modified in such a way that it takes less time, reducing overall assay time by more than 2 h.

2. Acceptability of HPV screening: Various approaches to self collection of samples for HPV testing are being explored in attempt to improve the accessibility of screening. The availability of a non cytologic screening method not requiring a vaginal speculum examination may reduce underscreening in women who have access to health care. A self collected screening method may also be expected to increase access to screening in may resource poor areas where there are limited numbers of clinicians trained in performing speculum examination. While a Canadian study concluded that this approach was acceptable to women and had sufficient sensitivity to warrant further evaluation, a South African study indicated that the technique was as sensitive as Pap, though less specific. In this study 66 per cent of all HSILS and cancer could be identified through HPV-DNA testing of a self collected vaginal sample, whereas 67.9 per cent would have been detected by a Pap smear alone.

However, there are some limitations to self collected samples. These procedure have a lower specificity than cytology screening and the sensitivity of HPV DNA testing of self collected samples is significantly less than that of HPV DNA testing of clinician obtained cervical specimens. There was only moderate agreement between the results obtained with HPV DNA testing of self collected vaginal swabs and clinician collected swabs (k=0.45).

Conclusions

As the problem of the developed world is to deal with high false negative rates of pap, the main emphasis has been on the liquid based cytology to reduce the sampling and interpretative errors and to use HPV screening as an adjunct so as to increase its sensitivity and NPV.

In developing countries, where no infrastructure has ever been created for cytology screening and barriers exist to raise necessary manpower, training
and maintaining quality control of cytology, the main emphasis is to explore alternative modes of screening including VIA/VILI or HPV. Though test characteristics and detection rates for different modalities have been adequately studied in several cross sectional studies and have been found to be comparable, the efficacy of the three tests differs widely\(^{17}\). Though one round of screening with VIA could successfully reduce incidence and mortality from disease\(^ {26}\), the Barshi trial failed to show any reduction in mortality or in number of advanced cancers in VIA and cytology arms. HPV was the only arm to have shown significant reduction in mortality within 8 years of one round of screening.

Some technical issues remain to be resolved for both VIA and HPV screening. For VIA, low specificity is a matter of concern, as also the case of uniform reporting of lesions. Since it is entirely a provider-dependent test training of paramedical is an important issue. For HPV screening, there is an urgent need for developing cheap, easy and a field test that can deliver results fast. In addition, treatment protocols need to be evolved especially for those who test positive for HPV but do not have any lesion.

Feasibility studies and demonstration projects will then be needed to know the infrastructural and operational requirements of screening based on visual inspection and HPV detection.

References


