Review Article

Screening and diagnostic modalities in carcinoma cervix: A Pathologist's perspective

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Keywords: *Carcinoma cervix; Pap smear; Liquid based cytology; Human papilloma virus; Screening.*

Abstract

Carcinoma cervix is the second most common cancer among women worldwide with majority of cases in the developing nations. It develops over a considerable period of time through precursor lesions that are amenable to detection when properly screened. A sea change has occurred in the detection and further the management of cervical cancer with the advances in the field of diagnostics. The orchestra ranges from the conventional Papanicolaou (Pap) test to high throughput expression profiling. The discovery of human papilloma virus (HPV) as the etiologic agent with recognition of various high risk types prompted the development of techniques for HPV detection. Several biomarkers have been recognized although many of them still require validation before they can be put to use at a large scale. Efforts directed at early detection of carcinoma cervix are desired for reducing incidence rates for carcinoma cervix. This review highlights the screening guidelines and the entire available armamentarium which can be applied to screen and diagnose cervical cancer at an early stage. However it can be foreseen that the etiology based testing is unlikely to replace cytology as a screening modality although it will remain a useful adjunct. This is especially true for the developing countries which are resource poor where cost-effective Pap test continues to be main method.

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Date of Submission: Sept 16, 2014 **Date of Acceptance:** Sept 20, 2014 **Date of Publishing:** Sept 28, 2014

How to cite this paper:
Mohan H, Kundu R. Screening and diagnostic modalities in carcinoma cervix: A Pathologist's perspective. Annals of Pathology and Laboratory Medicine. 2014;1(2):R1-R7.

Introduction

Carcinoma cervix is the second most common cancer among women around the globe and one of the lead causes of cancer deaths among females.[1] More than 80% of cases are found in the developing countries, particularly in Latin America, sub-Saharan Africa and India.[2] Human papilloma virus (HPV) having over 100 subtypes are found in 99.7% of females harboring the disease.[3] It is believed that at least 70% cases of cervical cancer can be prevented with the use of recently approved vaccines against HPV16 and 18.[4]

Many screening programmes have been introduced and Papanicolaou (Pap) smear based cytological screening programmes are commonplace in most of the developing countries. This for sure has resulted in a considerable decrease in the mortality rates. The limitation faced is the need for follow-up investigations due to low sensitivity of a single cytological examination confronted with the challenges of sampling and interpretative errors. The implementation of screening programmes is largely done at the health centers and outreach programmes targeting affected population are much desired. The need is highlighted by the fact that about 30–60 % patients newly diagnosed with cervical neoplasia never had a screening done.[5,6] Amongst those diagnosed with the advanced disease failed to undergo a screening test within the last 5 years. The inadequacy of follow-up after an abnormal Pap smear report is to the tune of 15 %.[6]

With the recent advances in the field of diagnostics, the armamentarium of cervical cancer screening and detection has expanded ranging from the conventional Papanicolaou test to high throughput expression profiling techniques which have revolutionized the detection and further the management of cervical cancer.

Clinical symptomatology and approach

Clinical presentation depends on the exact location and the disease extent of disease. The disease in the early stage is largely asymptomatic and detected on Pap smear. Symptoms include spontaneous or contact bleeding, pain, vaginal discharge and backache. Cervical biopsy is done for visible lesions with unaided eye. When colposcopy is unsatisfactory, or a high grade lesion is reported on Pap test or frank invasion cannot be ruled out on a colposcopic biopsy, conisation is done.[2]

The conventional Papanicolaou test

George Papanicolaou observed cancer cells in the vaginal smears of women with cervical cancer in the early twentieth century.[7] A milestone in the history of cytology was achieved in 1954 when Papanicolaou published his monumental monograph entitled ‘Atlas of Exfoliative Cytology’. The Pap smear is a safe, cost effective and practically non-invasive screening modality for cervical neoplasia. It is also an easy way to follow-up the cases with abnormal reports. With three annual consecutive screenings in an appropriate target population, the risk for missing serious disease is only about 1%.[8] Standardization of cytological terminology and better correlation with the histology reports is achieved with the Bethesda System of reporting Pap smears. This new system has further helped the clinician in management decisions.

Pap test has been quite effective for detecting squamous abnormalities but less effective in detecting cervical adenocarcinoma.[9] The sensitivity for detection varies from 48–91%.[10-13] Further, the sensitivity for detecting adenocarcinoma in situ ranges from 55–72%.[14] The false negative reports are largely limited to very well-differentiated adenocarcinomas like adenoma malignans and villoglandular adenocarcinoma.

From conventional cervical smear to liquid based cytology

Only about 20% of exfoliated cells obtained end up on a conventional Pap smear slide.[15] The conventional Pap smear has several limitations.[16] The procedure is difficult to standardize in view of manual application of cells to the glass slide, uneven distribution of cells onto the slide leading to overlapping cells resulting in erroneous interpretation at times. Obscuration of cells by mucous, blood or inflammatory cells is often problematic. Air drying artifacts are not uncommon. Further, conventional Pap smear has a problem of false-negative screening results. The liquid based cytology (LBC) has been quite successful in overcoming these challenges confronting the conventional Pap smear.

The LBC collection technique has improved both, the cytology sampling and specimen quality, enhancing the detection of precursor lesions of cervical cancer.[17] The cells are rinsed into a liquid preservative (CytoLyt for ThinPrep and CytoRich for SurePath) with immediate wet fixation of specimen which ensures better preservation. Residual specimen vials can be kept at room temperature for several weeks or months without a compromise on cell preservation or quality of slide preparation.[18-20] The material is also available for ancillary tests. There are, however, few disadvantages of LBC.[17,21] There is disruption of architecture with some cytologic alterations; breakage of papillae, cell groups and smaller cell and nuclear size. The

background material may be lost, reduced or altered.

Automated systems (computer assisted systems) for primary screening of Pap tests

In today's era, automated systems are available for screening which include ThinPrep imaging system (TIS, Hologic Corp., Marlborough, MA) and Focal point primary screening system (FPPS, BD Diagnostics, Burlington, NC) earlier known as AutoPap system. The screening of conventional smears is relatively insensitive. In a recent study by Yeong et al,[22] the abnormality pickup rate of 7.3% for ThinPrep imager (TPI) assisted screening and 7.8% for manual screening was reported. The rate of unsatisfactory smears reduced to half from 1.68% to 0.82% by imager assisted screening. The technique was found to be more sensitive for high grade lesions. The system allows up to 25% of the normal slides scanned to be sorted out without further human review.

DNA image cytometry

DNA image cytometry (DNA-ICM) is now a well accepted diagnostic adjunct for evaluating patients with cervical intraepithelial lesions and invasive cervical cancers.[23] With DNA aneuploidy values between 84% and 100% the positive predictive values for occurrence of in situ or invasive carcinoma from mild to moderate cervical dysplasias are high.[24-27] Kashyap and Bhambhani[28] reported increasing frequency of DNA aneuploidy from a well differentiated squamous cell carcinoma to moderately differentiated and poorly differentiated one. DNA-ICM also helps in the identification of malignant transformation in endocervical lesions.[29]

A study has also come up with the proposals for clinical consequences of DNA-ICM results:[23] 1) a high negative predictive value of 95% in cervical smears with atypical squamous cells with undetermined significance (ASCUS) and low grade squamous intraepithelial lesion (LSIL) diagnoses revealing DNA euploidy allows patients to return to normal screening intervals; 2) positive predictive values of 46% for patients who have cervical intraepithelial neoplasia (CIN) 3 or higher-grade lesions after 2 months and up to 100% after 3 years for patients who have ASCUS and LSIL with DNA aneuploidy allow the removal of lesions by conization or loop electrical excision procedure.

DNA-ICM is a fairly reliable method having a good reproducibility. Nguyen et al[30] have reported an interobserver correlation of 94.1% in DNA measurements done on 202 routine ASCUS- positive smears. A high value of interobserver agreement achieved may be due to a high standardization of DNA measurements and diagnostic data interpretation. DNA histogram interpretation is objective based on algorithms which are well defined.

Human telomerase RNA gene

The human telomerase RNA gene (hTERC) encodes the RNA component of the human telomerase. It is localized on chromosome 3q26, a region among the most frequent chromosomal gains in the cervical carcinogenesis.[31] Heselmeyer-Haddad et al[32] were the first to demonstrate hTERC amplification being common with an increase in severity of cervical lesions using fluorescence in situ hybridization (FISH).

Recently, Xiang et al[33] compared the amplification patterns of hTERC in invasive cervical carcinomas and CIN3. Copy numbers of the hTERC gene were measured by FISH. Nucleus with abnormal FISH pattern for hTERC was observed in 0.94–90.65% and 0–85.59% in squamous cell carcinoma (SCC) and CIN3 cells respectively. High level amplification was more common in SCC than CIN3. It was drawn that hTERC amplification was common in cervical exfoliated cells from SCC and CIN3 however clinical usefulness was limited in invasive cervical cancer.

Functional biomarkers

Functional biomarkers of precancerous lesions in cervical cancer include[34] $p16^{INK4a}$, Ki-67, p53, retinoblastoma protein (pRb), p21, p27, MCM5, CDC6, cyclin A, E and D which are cell cycle markers. Cytokeratins like CK14 and CK13 which are markers of squamous differentiation and other molecules like involucrin, telomerase, survivin, VEGF, FHIT, etc are also part of the orchestra. These biomarkers have an ability to distinguish CIN from non neoplastic lesions and also help in assessing the potential for progression or regression of CIN. $p16^{INK4a}$ and Ki-67 are the most widely available and used biomarkers.[35] In a recent study, it was envisaged that a negative or weak immunocytochemical p16 staining pattern, especially when combined with a positive L1 expression, may be a useful diagnostic indicator of a high grade lesion.[36]

Recently an immunocytochemical study on a relatively large number of LBC samples showed that TAp73 and p634A4 immunoreactivity correlated with subsequent detection of HSIL or above in patients with ASCUS and LSIL, respectively.[37] Further it was seen that cases of ASCUS positive for p634A4 were more likely to harbor high-risk HPV.

It was drawn that p634A4 and TAp73 may be useful potential biomarkers for triage of borderline and low grade cervical smears, respectively.

p16^{INK4a} immunocytochemistry

p16^{INK4a} is a cyclin dependent kinase inhibitor which regulates the activity of cyclin dependent kinases 4 and 6.[38,39] Marked increase in levels of p16INK4a is seen in HPV associated tumors due to inactivation of Rb by E7. Recently, a meta-analysis was carried out to assess the utility of p16^{INK4a} immunocytochemistry over HPV testing for triage of women with minor cytologic abnormalities.[40] The pooled sensitivity of p16^{INK4a} to detect CIN2+ was 83.2% and 83.8% for ASCUS and LSIL cervical cytology respectively. The pooled specificities were 71% and 65.7% respectively. p16^{INK4a} immunocytochemistry may be recommended for use in the triage of women with ASCUS. In LSIL triage, it is less sensitive but more specific than hybrid capture 2 and hence can be used as a first-step triage with further diagnostic workup of positive cases.

Expression profiling in carcinoma cervix

It is seen that cervical cancer has a differential gene expression. The gene profiles for adenocarcinoma and squamous cell carcinoma are different as seen with combination of cDNA microarray, real-time quantitative polymerase chain reaction and immunohistochemistry.[41] The distinction between cervical adenocarcinoma and an endometrial adenocarcinoma is difficult most of the times on endometrial curetting. Tissue microarrays enable a comparison of the immunoprofile of primary cervical and endometrial adenocarcinoma employing an antibody panel.

Human papilloma virus testing

High risk HPV testing is advocated by many groups including the American Cancer Society (ACS), the American Congress of Obstetricians and Gynecologists (ACOG) and the American Society for Colposcopy and Cervical Pathology (ASCCP).[42] The testing is useful in screening and for triage of results which are equivocal cervical cytology.

The US Food and Drug Administration (FDA) approved indications for testing high risk HPV are as under: [42]

1) Triage of patients with equivocal (ASCUS) cervical cytology results.

2) Adjunctive use along with cervical cytology in primary screening for patients over the age of 30 years.

3) Follow-up HPV testing for types 16 and 18 in patients over the age of 30 years who demonstrate an initial non type-specific high risk HPV positive result in the setting of a negative index cervical cytology result.

There are a host of molecular assays for detection of HPV besides the conventional cytomorphologic evaluation.[42,43] These include: Hybrid Capture II HPV (Digene), Cervista HPV HR (Hologic), Cervista HPV 16/18 (Hologic), CareHPV (Qiagen), Linear Array HPV Genotyping (Roche), Reverse Line Blot (Roche), PapilloCheck (Greiner Bio-One), INNO-LiPA HPV Genotyping, Amplicor HPV (Roche), RealTime HPV Assay (Abbott) and GenoID Real-Time HPV Assay.

Screening guidelines

The screening guidelines for carcinoma cervix are as under: [44]

1) For women aged 21 to 29 years: Screening with cytology alone every 3 years is recommended. HPV testing should not be used to screen women in this age group.

2) Women aged 30 to 65 years: Screening with cytology and HPV testing (cotesting) preferably every 5 years or with cytology alone every 3 years is acceptable.

3) Women aged older than 65 years: Should not be screened for cervical cancer with any modality if there is evidence of adequate negative prior screening and no history of CIN2+ within the last 20 years.

When three consecutive cytology results are negative or two consecutive cotests are negative within a span of 10 years before stopping screening and the most recent test occurring within the last 5 years it is called adequate negative prior screening.

Women with a history of CIN2, CIN3 or adenocarcinoma in situ following spontaneous regression or appropriate management of these should undergo routine screening for at least 20 years.

4) Women at any age who have undergone hysterectomy with no history of CIN2+ should not be screened for vaginal cancer.

5) Recommended screening practices are irrespective of the HPV vaccination status.

Clinical screening by visual inspection methods

In resource poor countries, clinical screening for cervical cancer can be done by visual methods like

visual inspection with acetic acid (VIA) and visual inspection with Lugol's iodine (VILI).[45,46] VIA is done using 5% acetic acid and appearance of acetowhite areas in the transformation zone is taken as positive. VILI positive is defined as no uptake of Lugol's iodine indicated by mustard yellow color while VILI negative is when there is an uptake indicated by development of brown color. VILI also known as Schiller's test was introduced way back in 1938.[47] The results of a recent cross-sectional study on 350 women subjected to Pap test, VIA, VILI and colposcopy are encouraging.[48] The VIA, VILI and Pap smear had a sensitivity of 89.5%, 100% and 52.6%, respectively while the specificity was 91.2%, 93.3% and 99.1%, respectively. The Latin American screening (LAMS) study, however, does not advocate the use of VIA and VILI as stand-alone tests but as combined tests with the Pap test or Hybrid Capture II for specific detection of cervical abnormalities.[49]

Follow-up based on screening results

1) Women with HPV positive and cytology negative cotests: Repeat cotesting in 12 months or immediate HPV genotype specific testing for HPV16 alone or for HPV16/18.

1a) Women who are HPV positive or have LSIL or more severe cytology on a repeat cotesting should undergo colposcopy.

1b) Women testing positive for HPV16 or HPV16/18 on immediate HPV genotype specific testing should undergo colposcopy. Women testing negative for HPV16 or HPV16/18 should be cotested in 12 months.

2) Women who are HPV negative and have ASCUS or negative cytology should undergo routine screening.

Conclusion

Efforts directed at early detection of carcinoma cervix are desired for a fruitful outcome. Despite availability of a large number of detection methods for HPV, the etiology based testing does not seem to replace cytology as a screening modality in reducing the incidence rates for carcinoma cervix. This is more so true for the developing nations where there exist cost constraints and Pap test is economical and easily available.

Acknowledgements

None.

Funding

None.

Competing Interests

None declared.

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