Review Article

The Role of Biomarkers (p16^{INK4a} and Ki-67) in Cervical Cancer Screening: An Appraisal

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Abstract

Cervical cancer is a common and important public health problem in developing countries. Even though many developed countries have achieved significant successes in reducing invasive cervical cancer burden, the burden in developing countries is still worrisome. Currently, cervical examinations and Papanicolaou (Pap) tests remain the screening method of choice for most women in many parts of Africa. Molecular diagnostic tests for human papillomavirus (HPV) can augment screening for cervical cancer when used in conjunction with the Pap smear. Due to the inherent limitations of morphologic interpretation and borderline cases, the atypical squamous cells (Atypical Squamous Cells of Undetermined Significance [ASCUS] and Atypical Squamous Cells cannot rule out High Grade [ASC-H]) were introduced and this led to significant colposcopic follow-up and/or treatment of these women. p16^{INK4a} is an efficient triage method; the dual staining with Ki-67 was introduced mainly to increase reproducibility and specificity compared with stand-alone p16^{INK4a} staining. Diffuse p16^{INK4a} immunostaining is the hallmark of high-grade squamous intraepithelial lesions regardless of HPV status.

Keywords: Appraisal, cervical cancer screening, immunocytochemistry, Ki-67, p16^{INK4a}, triage

Introduction

Cervical cancer is a common and important public health problem for adult women in developing countries.[1,2] Many industrialized countries have achieved significant successes in reducing invasive cervical cancer burden over the past six decades and with annual incidence rates between 4 and 14/100,000.[1] In developed countries, an estimated 15,000 new cases of cervical cancer and 5000 deaths occur annually from the disease.[3] These relatively low figures are a far cry from those obtainable in Nigeria where an estimated 250/100,000 cases and 155,000 deaths are recorded annually. Globally, women particularly those living in developing countries suffer higher rates of morbidity and mortality from cervical cancer than previously noted.[3]

Cervical cancer no longer ranks among the top ten cancers in these settings. The low incidence is achieved through substantial healthcare investments for screening programs and diagnostic workup in these countries. On the other hand, cervical cancer is the leading cancer among women in many resource-constrained settings of the developing countries, where incidence and mortality rates are about five to six times higher.[4] Rates are highest in sub-Saharan Africa, South East Asia, and parts of South America, where cervical cancer represents from a sixth up to a fifth of all cancers among women.[1] Cervical cancer is not only the second most common malignancy in adult women but is also the most common malignancy of the female genital tract in Nigeria.[5,6]

The value of screening for cervical cancer has been proven.[1] Such screening in developed countries has achieved a decrease in incidence and mortality by about 80%.[7] Although screening facilities are available in many parts of developing countries, the incidence of cervical cancer remains very high, and many patients present with late stage disease.[8] Detecting the high-risk human papillomavirus (HR-HPV) DNA is more sensitive test for cervical cancer early detection than Papanicolaou (Pap) cytology. It is used in addition to Pap

Access this article online

Quick Response Code:

Website: www.atpjournal.org

DOI: 10.4103/atp.atp_3_17

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How to cite this article: Ahmed SA, Obaseki DE, Mayun AA, Mohammed A, Rafindadi AH, Abdul MA. The role of biomarkers (p16^{INK4a} and Ki-67) in cervical cancer screening: An appraisal. Ann Trop Pathol 2017;8:1-4.

cytology in certain settings and currently introduced as an alternative for primary screening in many industrialized countries.[9] However, considering the high prevalence of HPV infections particularly in young women, detecting HPV-DNA is a poorly specific test for real cellular alterations and therefore requires additional triage tests for the specific identification of women needing further workup or treatment.[10] To reduce referral rates, unnecessary treatments, and finally costs, new biomarkers (p16INK4a and Ki-67) have been identified and suggested to improve diagnosis of cervical cancer and its precancerous lesions.[11,12]

Risk Factors for Cervical Cancer

Known risk factors for cancer of the cervix include persistent infection with HR HPV, early age at first intercourse, and multiple sexual partners. A male consort who in turn has had intercourse with multiple women also confers a significant risk.[13] Other risk factors of cancer of the cervix include cigarette smoking and immune suppression, especially those who have undergone renal transplantation. HIV infection may increase a women's risk for cervical neoplasia. It is claimed that the vast majority of cervical cancers could be prevented if all women were offered and complied with high-quality cytological screening programs.[14]

Human Papillomavirus in Cervical Cancer

HPV is the most prevalent sexually transmitted infection in the world, occurring at some point in up to 75% of sexually active women. Nearly, all cervical cancers (99.7%) are directly linked to previous infection with one or more of the oncogenic types of HPV.[1]

Currently, there are >100 different known HPV genotypes that have been grouped into low-risk and high-risk categories and designated as causing mucosal or cutaneous infections.[15] Warts are generally the result of infection by low-risk types of HPV, including 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and CP6108. High-risk types of HPV include 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82. The high-risk strains induce cervical dysplasia and can lead to the development of several types of cancers including cancer of the cervix, vulva, vagina, anus, and penis. The most common of these is HPV-associated cervical cancer.[16] HPV types 16 and 18 are the most prevalent worldwide.[17]

The viral E6 and E7 oncoproteins are necessary for malignant conversion. The abilities of high-risk HPV E6 and E7 proteins to associate with the tumor suppressors p53 and retinoblastoma protein (pRB), respectively, have been suggested as a mechanism by which these viral proteins induce tumors. The E7 proteins encoded by the high-risk type HPVs bind Rb with a much higher affinity compared to those encoded by the low-risk type HPVs. One of the major biochemical functions of Rb is to bind E2F-family transcription factors and repress the expressions of replication enzyme genes.[5] E7 disrupts the interaction between Rb and E2F, resulting in the release of E2F factors in their transcriptionally active forms.[6] This E7-mediated conversion of E2Fs to their activator forms stimulates replication and cell division, which is consistent with the observation that keratinocytes constitutively expressing E7 remain replication competent, even after differentiation.[7] Due to the high prevalence of HPV infections in younger women, HPV testing currently is not recommended for screening women younger than age 30 years.

Cervical Cancer Screening

Pap stain is the gold standard for detecting abnormal cervical epithelial cells, using microscopic analysis of conventional cervical smears or cell suspensions from liquid cytology medium. Currently, cervical examinations and Pap tests remain the screening method of choice for most women in many parts of Africa. Morphological findings from a cytology analysis determine the level of risk for developing cervical malignancy. The efficacy of the Pap test, however, is hampered by high interobserver variability and high false-negative and false-positive rates.[1-3] This is obviated by repeated screening at frequent intervals thus ensuring a high level of detection and protection. Molecular diagnostic tests for HPV can augment screening for cervical cancer when used in conjunction with the Pap smear.[18]

The cervical cytology is reported using the Bethesda system of classification which established the 2-tiered reporting system for squamous intraepithelial lesions (SILs): low-grade SIL (LSIL) (cervical intraepithelial neoplasia [CIN] I) and high-grade SIL (HSIL) (CIN II and CIN III). This terminology reflected the up-to-date understanding of HPV biology – squamous epithelium is affected by the virus in essentially two ways: either as viral infection or as viral-associated precancer.[19,20] Due to the inherent limitations of morphologic interpretation and borderline cases, the atypical squamous cells (Atypical Squamous Cells of Undetermined Significance [ASCUS] and Atypical Squamous Cells cannot rule out High Grade [ASC-H]) were introduced and this led to significant colposcopic follow-up and/or treatment of these women.[20,21]

Role of biomarkers (P16 and Ki67) in the management of abnormal smears

Various immunocytochemical markers have been evaluated with respect to their specificity in staining dysplastic cells either in biopsies or in cytological smears.

Colposcopy is usually done on women with abnormal smears and/or positive HR-HPV test. However, a newer concept of triage using biomarkers $p16^{INK4a}$ and Ki-67 dual immunostaining has been advocated to avoid over referral for colposcopy. $P16^{INK4a}$ is an efficient triage method; the dual staining with Ki-67 was introduced mainly to increase reproducibility and specificity compared with standalone P16INK4a staining.[22] Diffuse $p16^{INK4a}$ immunostaining is the hallmark of HSIL (CIN2 and CIN3), regardless of HPV status.[23]

The overexpression of the cyclin-dependent kinase inhibitor p16INK4a (p16) in cervical dysplasia has been shown to be associated with the transforming activity of the E7 oncoprotein of high-risk HPV types, and it is a surrogate marker of the E7-mediated inactivation of the tumor-suppressor function of the pRb. In replicating cells, the transcription factor E2F is regulated by phosphorylation of RB. Rb phosphorylation is normally mediated by cyclin-dependent kinases (CDK4, CDK6) that are controlled by several kinase inhibitors (INKs). Aberrant expression of E7 in basal cells disrupts binding of pRB to E2F that is counteracted by massive expression of p16INK4a, an important CDK inhibitor. Since E7-dependent E2F release is not mediated by phosphorylation of Rb, the counter-regulatory p16 INK4a expression has no effect on the activated cell cycle. Nondysplastic epithelia infected with LR- or HR-HPV do not diffusely stain for $p16^{INK4a}$. In sharp contrast to this expression pattern of p16INK4a in resting cells with aberrant differentiation, the pathological expression in HPV transformed cells is indicated by a very strong diffuse staining pattern in the replicating cells of the basal and parabasal cell layer. Basically, all cervical carcinomas, CIN3 lesions, as well as the majority of CIN2 lesions are diffusely positive in immunohistochemistry.

Proliferation-associated antigens such as Ki-67 are related to DNA replication and specifically highlight cells with active DNA replication.[16-21] Since HPV infection leads to increased epithelial cell proliferation in infected tissues, increased Ki-67 staining can be an indicator of HPV infection. In normal human cervical squamous mucosa, expression of Ki-67 is limited to the proliferating basal and parabasal cells. In dysplasia and carcinoma, however, expression extends above the basal one-third of the epithelium and the number of positive cells increase, with a significant positive correlation between ascending grade of SIL and labeling index.

Normally, the p16 protein triggers cell cycle arrest in the course of cellular differentiation processes and is rarely observed simultaneously with Ki-67. However, in transforming HPV infections, p16 is strongly overexpressed in proliferating cells. Observing dual expression, therefore, suggests HPV-induced deregulation of the cell cycle and may be used as an indicator for the presence of high-grade lesions.[24]

The workup of the primary test result (i.e., the triage) can be the second of application for novel biomarkers. Currently, HPV testing is recommended as one option to triage ASC-US cytology.[25] Biomarkers used in triage should be specifically associated with disease progression. Some novel biomarkers such as $p16^{INK4a}$ and Ki-67 have been evaluated in comparison with HPV testing and other markers.[26] In a multicenter study in China, it was found that the p16/Ki-67 positivity increased with histologic severity, and the sensitivity and specificity of p16/Ki-67 to detect CIN2+ in the entire population were 90.9% and 79.5%, respectively. In women with ASC-US and LSIL, sensitivity and specificity for detection of CIN2+ were 87.5% and 66.4%, respectively. Therefore, p16/Ki-67 dual-stained cytology provided a high sensitivity and moderate specificity to detect underlying cervical precancer and cancers in various settings and might be considered as an efficient screening tool in screening.[27]

Conclusion

In addition to primary and triage screening markers, biomarkers could be used for a risk assessment of detected lesions, to stratify intermediate lesions, to predict progression, and to monitor recurrences after treatment. A very interesting field for biomarkers could be the assessment of LSIL and borderline lesions.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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