The Role of Human Papilloma Virus (HPV) Testing in Cervical Cancer Screening

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ABSTRACT

Background: *Cervical cancer is the most studied gynaecologic cancer with a clear natural history. Screening for the premalignant lesions has reduced the mortality from cervical cancer mainly in developed countries. Cancer of the cervix is regarded as a sexually transmitted disease because of its association with human papilloma virus. The backbone of screening has been pap smear. The variability of the sensitivity and specificity of Pap smear has lead to development of other screening tests including HPV testing.*

Aim: *To review the role of human papilloma virus testing in cervical cancer screening.*

Methodology: *A medline and other internet search engines were accessed to retrieve online publications on human papilloma virus and cervical cancer. Textbooks and other hard copies of publications on human papilloma virus and cervical cancer were also accessed and information extracted.*

Result: *HPV testing can be used either alone or as an adjunct screening test for pre-malignant lesions of the cervix. It can also be used in monitoring of treatment. It provides an explorable option in low resource countries with high disease burden and no organized screening programme.*

Conclusion: *Development of strategies that will incooperate HPV testing will reduce the false positive results from pap smear and increase the uptake of cervical cancer screening in developed countries. The extent of the role will also be determined by the existing infrastructure.*

Key words: *Cervical cancer, screening, human papilloma virus, testing.*

INTRODUCTION

Cervical cancer is largely a preventive disease that is preceded by a long pre-invasive lesion. The disease is both preventable and curable if detected early. The mortality associated with cervical cancer in developing countries is very disheartening. Udigwe et al in Nnewi, South east Nigeria recorded cervical cancer to account for 61.4% of all gynaecological malignancies[1]. Similar findings have been noted in other studies [2-5].It remains a leading cause of mortality. This high mortality is largely because of late presentation and diagnosis. There is no standardized strategy for primary and secondary prevention of cervical cancer in developing countries. In contrast, the prevalence and mortality of invasive cancer in developed countries has reduced drastically. This is because of organized screening methods that tend to diagnose and treat the pre-invasive lesions[6-12].

The major approach to cervical cancer prevention has traditionally focused on screening women for precancerous lesions using papanicolaou smears and treating the lesions. Pap smear was developed and named after the inventor Dr. George Papanicolaou. This makes use of exfoliative cells of the cervix to detect dysplastic or pre-invasive cells.

A pap smear is a cytological test designed to detect abnormal cervical cells. The procedure involves scrapping cells from the squamo-columnar junction. These collected cells are smeared onto a slide, fixed and stained by Papaniculaou method. Cervical cytology is considered very specific test for HSIL. A specificity of about 90% has been reported[13]. It is probably the most effective cancer screen test yet devised.

The prevalence of abnormal cervical smear varies depending on the study population and individual characteristics. Mbamara et al reported a prevalence rate of 29%[14]. A rate of 12.2% was reported from Enugu[33].This is higher than 8.4% reported from Ibadan[16]. Anorlu et al reported 5.0% in Lagos[17]. This is comparable to the value of 54 per 100,000 reported in Zimbabwe [18].

Despite its success, Pap smear has its shortcomings. One of the pitfalls is the low sensitivity of about 50-60% [19].This implies high false negative rate that may have medical, financial and legal implications. One of the major challenges of Pap smear is the high level of unsatisfactory Pap smear. This is mainly due inadequate sample collection. Interpretation and management of borderline or abnormal squamous cell of undetermined significance may also pose management challenge.

In USA, a study noted ambiguous Pap smear result in 3 million out of 55 million women[20]. Meta analysis has shown that it will be very difficult to achieve high sensitivity and specificity with pap smear[21]. Classification of Pap smear and follow up biopsies is subject to high inter-observer variability. Liquid based and automated cytology are method devised to reduce

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sampling errors[22-24]. However, they do not remove the errors completely.

Human papilloma virus test has been described as an important strategy for improving quality of screening for prevention of cervical cancer.

The HPV testing is performed by reacting denatured single stranded DNA with RNA probes of the oncogenic HPV types. Hybrids consisting of target HPV bound to RNA probes are bound or caputured on sides of tubes coated with antibodies recognizing DNA:RNA hybrids. Adding a second antibody tagged with alkaline phosphatase will permit detection of bound hybrid by a chemoilluminescent readout. Positive test specimen is one in which light emission (expressed in relative light units RLUs) is equal to or greater than the mean of positive controls.

HPV testing can be applied as primary screening tool, as a triage of atypical squamous cell abnormality and as a test of cure. Rapid testing of HPV appears to be an attractive option for cervical cancer screening especially in resource poor countries with no organized cancer screening program. This is based on the strong association of HPV as a prime aetiological agent in the pathogenesis of cervical cancer. This article will review the merits and demerits of HPV testing in cervical cancer screening.

HPV TESTING AND SCREENING OF CERVICAL CANCER

Testing for HPV virus is premised on the association of high-risk HPV subtypes in the aethiogenesis of pre-invasive and invasive cancers of cervix. Human papilloma viruses are the prime aetiologic factor in the development of cervical intraepithelial neoplasia and cervical cancer. HPV is present in 80% of all CIN lesions and in 99.7% of all invasive cancers[25-26].Transmission of HPV infection is by close contact.

About 71 different genotypes have been identified[27]. The HPV that infects anogenital region is divided into two broad groups based on their malignant potential. The low risk group includes HPV 6 and 11 while the high-risk group includes HPV 16, 18 and 45.

E6 and E7 early proteins have been implicated in the pathogenesis of CIN and cervical cancer[26-28]. E7 binds and inactivate retinoblastoma (Rb) proteins while E6 binds and inactivates the P53 protein. This leads to functional loss of P53 and RB growth suppression function. There is resultant resistance to apoptosis causing uncensored growth after DNA damage, which may cause malignancy. Human papilloma virus alone is not enough to cause cancer.[1]

More than 90% of HPV infection on immmunocompetent individuals will regress spontaneously over 2 years[26-27]. Only about 3- 5% will have cervical abnormalities CIN lesions are clinically heterogeneous lesions. They can regress, persist and occasionally progress to cancer of the cervix. Rates of regression of CIN lesions vary depending on the grade of the lesion. About 50-90% of CIN I regresses while 40% and 30% of CIN II and CIN III respectively regress[30].

It has been established that risk factors for progression of HPV infection to CIN and cervical cancer include the HPV serotype with HPV serotype 16 and 18 being more associated with cervical cancer[31-34]. Other risk factors include persistent infection, immunosuppression, smoking and vitamin deficiencies. Age at first sexual intercourse and number of sexual partners significantly increase risk of cancer[30].

HPV TESTING AS A PRIMARY SCREENING TEST

HPV testing has been found to be a good adjuvant test to Pap smear in screening for cervical cancer. Combination of HPV and cytology test improves the sensitivity of the screening. This decreases the false negative rate. Studies have noted that double negative test (Negative HPV test and Pap smear test) is a better prognostic assurance against the future risk of developing CIN III or HSIL than three subsequent negative cervical smears[35]. This may allow for reduced or spacing of screening test.

The Canadian Cervical Cancer screening Trial (CCCaST) compared the sensitivity and specificity of HPV testing with conventional Pap smears in 10,154 women aged 30-69 years who underwent both tests[36]. Colposcopy was carried out for women with abnormal Pap smear and positive HPV test. Sensitivity for detection of confirmed CIN grade 2 or worse was higher with HPV testing than with conventional Pap smear. However, the specificity was lower. When both sensitivity and specificity of both tests were estimated, none was superior.

The Population Based Screening Study Amsterdam (POBASCAM) trial analysed data on 17,000 women aged 30-59 who were randomly assigned with conventional Pap plus HPV (intervention group) and Pap smear alone (control)[37]. These women were re-screened after five years (the interval for routine screening in Netherlands) and both arms of the study underwent Pap smear and

HPV testing. CIN 3 or worse lesions were detected more in the intervention group than control at the initial screening. The second screening after five years showed that fewer women in the intervention group had CIN 3 or worse. This shows that HPV testing led to earlier detection of the lesions.The overall rate of CIN 3 or worse lesions were similar after the two rounds of testing. However, the effect on mortality was not determined by the study.

Katki et al in a randomised controlled trial, assessed the efficacy of cytology screening versus HPV in reducing the incidence of high grade lesions and invasive cancer in women aged 25-60 years[38]. This study showed that initial screening for HPV with or without cytology reduced the occurrence of invasive cancer of the cervix at second round of screening more than with cytology alone. The sensitivity of HPV testing in detecting CIN grade 2 or more was higher than cytology in the two rounds of testing.

The use of HPV test alone as the screening test forcervical cancer is not yet established. Despite its high sensitivity, it has a reduced specificity when compared to Pap smear. This implies that many young women with transient HPV infection will be subjected to unnecessary colposcopy examination. The long-term consequences are uncertain. Further studies will be needed to make a conclusion on the role of HPV testing as a primary screening test. In addition, there is no consensus on the algorithm for management. This has to be determined to help in comparison of result and effectiveness of the screening process.

HPV TESTING AS A TRIAGE

HPV testing has been found to be effective in triaging of women with inconclusive cytology result[39]. This means that when cytology is inconclusive, HPV testing can help determine whether the woman should be referred for immediate colposcopy or further surveillance. Patients with negative HPV results are returned to routine testing while HPV positive women are referred to further evaluation with colposcopy. HPV testing can also be used to resolve discordant cytology, colposcopy and histological findings.

The Trial of Management of Borderline and Low Grade Abnormalities (TOMBOLA) study showed that single HPV test in a women 40 years and above could help in decision making concerning further screening after cytology for low grade lesions[40]. However, it was not useful for women below 35 years. American College of Obstetricians and Gyneacologists (ACOG) has stated that HPV testing in adolescents is unnecessary because up to 80% may be HPV positive which is transient in most cases. The ASCUS-LSIL Triage study (ALTS) found that immediate colposcopy was the preferred strategy following a cytological diagnosis of ASCUS and LSIL[42]. In addition, the authors opined that HPV triage is a promising option for ASC-US.

There are various protocols for the management of women with ASCUS. The options include to (1) repeat cytology in 4-6 months (2) to refer for colposcopy immediately (3) HPV DNA testing for high grade type. The commonly utilized option is that of reflex HPV testing[43]. Those with a positive HPV DNA for high risk viral types are referred for colposcopy. Also, those with repeat abnormal cytology are referred for colposcopy. Those in whom no abnormality is found are screened with HPV DNA testing in 12 months or with cytology at 6-12 months.

The main advantage of HPV testing in this group is that the referral of about 50% of those with borderline smears can be eliminated. This will reduce the rate of unnecessary colposcopy. However high rate of HPV positivity in young women could lead to unnecessary colposcopy.

HUMAN PAPILLOMA VIRUS AS A TEST OF CURE.

The possible role of HPV testing as a test of cure is being evaluated. The basis is the negative predictive value of the test. In management of premalignant lesions of the cervix, complete excision of the lesion may lead to negative HPV test in 6 months[44]. Incomplete excisions tend to remain positive. There is need for more studies to determine the role of HPV testing in evaluation of cure.

HPV TESTING IN RESOURCE POOR COUNTRIES

HIV testing has a potential to improving the cervical cancer screening in developing countries. These countries have no organized cervical cancer screening programs which account for the high disease burden. Several diseases like malaria, tuberculosis, HIV, etc are competing for the limited resources. The critical issues of maternal and child mortality are yet to be addressed in these countries. The infrastructures are very poor making means of transportation and electricity to be a privilege instead of a norm.

Cervical cancer screening program in low resource settings must factor in these challenges. Such program should address most of these limitations. These include ability to perform the test in primary and secondary health facilities, ability of general practitioners to carry out the test. This must also require limited technology and staff training. In addition, test result should be either immediate like visual inspection of the cervix or be available within several hours.

The rapid test HPV offers an attractive option for screening in developing countries. The major drawback is the cost. This may be overcome by development of rapid tests for HPV, as was done for HIV screening. Studies in India have demonstrated that HPV testing reduces the cervical cancer mortality in developing countries and has been found to be superior to VIA or cervical cytology[39].

Two types of HPV tests available are the Hybrid Capture 2 and Cervista HPV test. HPV testing has a high sensitivity and low specificity. The low specificity of HPV testing poses a great management challenge in developing countries. Tests in developing countries requires high specificity to reduce need for further evaluation. In addition, immediate treatment for a test with low specificity may lead to unnecessary treatment. The specificity of HPV test can be increased by changing the definition of a positive test. This can be done by changing the positivity threshold, expressed as the ratio of light emission per positive control.

FUTURE OF HPV TEST IN CERVICAL CANCER SCREENING

Currently, a lot of research work is going on in isolation of molecular markers that can predict progression of HPV infection to pre-malignant lesions. Surrogate protein makers have been described. These include K1-67 and P16/Ink$_{4a}$ [41,46].The development of rapid test for HPV will scale up the cervical screening in developing countries. This will reduce the time, cost and skill needed to carry out the test. Currently, CareHPV, a type of rapid HPV test is undergoing trial in the USA[47].

CONCLUSION

HPV testing and application in management of cervical cancer has revolutionized the screening and management of cervical intraepithelial lesions. Its role in primary screening is not yet be defined. However, reflex HPV testing reduces the rate of unnecessary colposcopies.

Identification of molecular markers and other predictors of disease progression will be a major breakthrough in the management this disease. More research is needed to make conclusive deductions on the specific role of HPV testing in cervical cancer screening.

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