Low risk and high risk human papillomaviruses (HPVs) and cervical cancer in Zimbabwe: epidemiological evidence

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Abstract

Objective: To establish the prevalence of detectable low-risk and high-risk, oncogenic HPV types in cervical swabs of women with histologically proven cancer of the cervix.
Design: Cross sectional study.
Setting: Harare Central and Parirenyatwa Hospitals.
Subjects: 119 women with histologically proven cervical cancer of whom 63 had the degree of differentiation of the tumour reported.
Main Outcome Measures: Frequency of infection with high and low-risk human papillomaviruses.
Results: The presence of HPV DNA was demonstrated in 63% (75/119) of cases. Low risk HPVs were present in 26% (31/119) and high-risk HPVs were demonstrated in 51% (61/119) of samples tested. Co-infection with both low-risk and high-risk HPVs was observed in 14% (17/119) of the specimens. High-risk HPVs were detected in 55% (21/38) of poorly differentiated tumours while 60% (15/25) of moderately and well-differentiated tumours showed the presence of high-risk HPVs.
Conclusion: High-risk human papillomaviruses are associated with cervical cancer. There was no significant difference in the frequency of high-risk HPV types in women with moderately to well-differentiated tumours and those with poorly-differentiated tumours.

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Introduction

Cancer of the uterine cervix is one of the commonest cancers affecting Zimbabwean women. The malignancy which seems to be increasingly affecting younger women may become a major public health concern.[1] Persistent infection with HPVs, particularly the high-risk groups appear to be essential for cellular transformation into the various grades of cervical intraepithelial neoplasia (CIN) and established squamous cell carcinoma.[2] It is argued that

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cervical cancer does not occur in the absence of HPV infection. North American experience suggests that HPV DNA can be detected in 99.9% of cervical cancers.[3] At least 17 high risk HPV types which include types 16, 18, 31, 33, 45, 51 52 and 56 have been associated with cervical cancer.[4]

Two techniques are currently in wide use for the detection of HPV DNA: the first and second generation Digene Hybrid Capture (HC)[5] assays and Polymerase Chain Reaction (PCR)[6] targeted to conserved portions of the HPV genome. PCR is qualitative and semi-quantitation is possible. HC assays also yield qualitative data reflecting presence of HPV types in cervical epithelium extracts or biopsies. For screening purposes the commercial HC assays are superior to PCR due to reduced costs, semiautomation and quality control.[5]

Several reports have shown that detection of high levels of oncogenic HPVs has high predictive value for subsequent diagnosis of CIN.[6-12] It appears, therefore, that the addition of HPV testing to routine cytology can substantially increase the detection rate of high grade CIN with acceptable positive predictive value.[6] The Hybrid Capture I (HC I) assay kit includes probes for nine high-risk HPV types that have been found to be clinically relevant in European and North American studies. This test and the improved Digene Hybrid Capture II, assay which detects more high-risk HPV types are now commercially available and may be practical solutions to screening programmes in Zimbabwe.

It was, therefore, of interest to us in the present study, to use the first generation HC I tube assay to screen for HPVs in known cancer cases from Zimbabwe. The specific objective of the study was to determine if there is any association between the presence of high-risk HPVs types and the degree of differentiation of cervical carcinoma.

Materials and Methods

Patients.

A total of 119 consecutive women had their cervices swabbed at the time of speculum examination. All the women had histologically proven cancer of the cervix. Sixty three of these women had the stage of differentiation of the tumour reported. Cervical swabs were obtained by an experienced nurse or obstetrician and gynaecologist (one of the authors, GAS) and placed in 10 mls of Digene transport medium (Digene Corporation, USA).

All specimens were stored at -200°C in the short term or at -800°C for long term storage.

Detection of Oncogenic and Non-oncogenic HPVs.

Principle of the Hybrid Capture I Assay: The assay uses an HPV RNA probe B cocktail containing type-specific probes for the high-risk HPV types 16, 18, 31,33, 35, 45, 51, 52, and 56 and the HPV probe A cocktail containing RNA probes for the low risk HPV types 6, 11, 42, 43 and 44. The HC I technique is a solution hybridization antibody-capture assay that uses chemiluminescent detection. Specimens containing the target DNA hybridize

with a specific HPV RNA probe from a cocktail of probes. Resultant RNA-DNA hybrids are captured onto the surface of a tube coated with antibodies specific for RNA-DNA hybrids. Immobilized hybrids are reacted with alkaline phosphatase-conjugated antibodies specific for the RNA-DNA hybrids and detected with a chemiluminescent substrate. Emitted light is measured as relative light units (RLUs) on a luminometer (DCR-1 Luminometer, Digene Corp USA).

The assay was performed using the commercial Digene Hybrid Capture System I, according to the exact instructions of the Manufacturer (Digene Corp, USA). An RLU measurement which was equal to, or greater than the Cutoff Value indicated the presence of HPV DNA sequences in the specimen.

Results

Demographic Features.

The demographic details of the study subjects are summarized in Figure I. The mean age of the women was 48 years and the youngest patient was 22 years of age whilst the oldest was 71 years. The majority of the women (25%) were in the 36 to 45 year age group. Two patients were in the under twenty five age group.

Figure I: Age distribution of the cervical cancer patients.
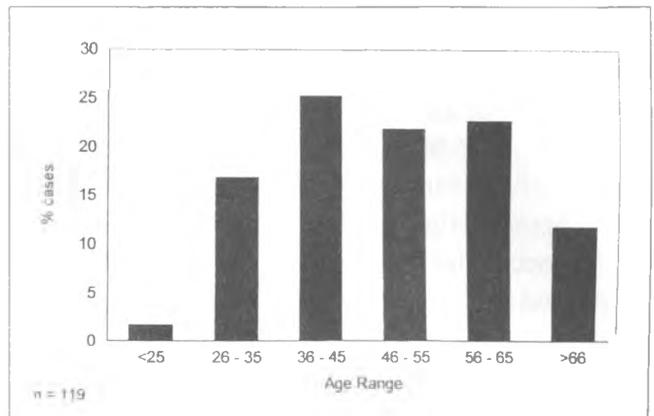
HPV-DNA in Cervical Swabs.

HPV DNA was detected in 63% (75/119) of cervical swabs. The high-risk HPV types were detected in 51% (61/119) of all samples examined. Low-risk HPV types were detected in 26% (31/119) whilst dual infection with both high and low-risk HPV types was shown in 14% (17/119) of the patients.

HPV Types and Differentiation of Cervical Cancer Lesions.

The degree of differentiation of tumours was established in 63 cases. Of these 38 were poorly differentiated, 22 moderately and three well-differentiated squamous cell carcinoma. As shown in Table I, high-risk HPVs were detected in 21/38 (55%) poorly differentiated tumours, 13/22 (59%) moderately differentiated and in 2/3 (66.7%) well-differentiated tumours.

When the women with moderately differentiated tumours are combined with those with well differentiated tumours (60% [15/25]), there is no significant difference in the frequency of high-risk HPVs compared to those with poorly differentiated tumours (55% [21/38]) χ^2 =0.01, p=0.911.

Table I: High risk HPV types and differentiation of cancer lesion.

Differentiation of cancer lesion	No. of cases n=63	No. positve for high-risk HPV	% positive for high-risk HPV
Poorly differentiated	38/63	21/38	55
Moderately differentiated*	22/63	13/22	59
Well differentiated*	3/63	2/3	67

* There is no significant difference in high-risk HPV frequency rate between women with moderately differentiated and well-differentiated tumours (15 25) compared to those with poorly differentiated tumours (21/38). Chi-square-test (X2=0.01, p=0.911)

Discussion

This is the first study to look at the association between HPV types and the degree of differentiation of cervical cancer in Zimbabwean patients. Half the patients with cervical cancer that were examined, were HPV positive for any of the high-risk types 16,18,31,33,45,51,52 and 56. In a study in Cape Town, South Africa, Williamson et al[13] tested 68 cervical biopsies for HPV DNA by PCR.

Eighty one percent of the cancers had detectable HPV-DNA while 25% of the biopsies contained HPV types other than types 16,18,31, 33 or 45. Factors, which might have contributed to the lower detection rate in our study may include the following:

(i) the relative insensitivity of the HC I assay compared to PCR and Southern-blot analysis,

(ii) the presence of oncogenic HPV types other than those present in the HC I assay probe mix,

(iii) the use of probes other than the type-specific HPV probes used in the other studies,

(iv) the use of cervical swabs rather than cervical biopsies as in other studies,

(v) the presumptive presence of yet uncharacterized HPV types in the study population.

Using *in situ* hybridization, it has been shown that the presence of oncogenic HPVs is strongly associated with the degree of differentiation of the cervical tumour.[15] The study by Womack et al[8] showed a strong link between high-grade squamous intraepithelial lesions (HGSIL) and the prevalence of high-risk HPVs in both (human immunodefiency virus) HIV infected and HIV seronegative women. Although our study did not investigate the HIV status of the patients, the results, although derived from a smaller sample are not in agreement with this observation. Geographical differences in the distribution of HPV types are well-documented.[10] Such differences are expected to be found within the African continent. The recognition of the central role that HPVs play in the pathogenesis of cancer of the uterine cervix has implications for the screening and prevention of this malignancy.

Further studies are currently underway in our laboratory to identify HPV types that are clinically important in our population. Such information will be important for designing population-tailored diagnostic reagents for screening purposes and for rational design of vaccines.

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