

Human papillomavirus DNA testing on self-collected vaginal tampon samples as a cervical cancer screening test in a Gauteng population

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Abstract

Background: There is a need to simplify cervical cancer screening to reach more women. Tampon-collected specimens can be tested using molecular methods, but this type of self-screening has not been properly evaluated as a screening method in South Africa before. The objective of this study was to evaluate human papillomavirus (HPV) DNA testing of self-collected tampons as a screening method in an urban and peri-urban population in Gauteng by comparing the results with the current standard of conventional cytology. In addition, HPV prevalence, type, distribution and incidence of cytological abnormalities in this population are described.

Method: Seven hundred and twenty women attending public healthcare facilities in and around Tshwane, Gauteng province, were invited to participate. The women collected a tampon sample for molecular testing, and were then screened by healthcare workers collecting a conventional cervical cytology smear. HPV testing was undertaken using the Linear Array[®] HPV Genotyping Test (Roche Molecular Systems).

Results: Data for analysis were available for 631 women. Three hundred and fifty-four (58%) were positive for high-risk HPV, while (15.4%) had an abnormal cytology result. Women aged 30-39 years had the highest prevalence of both high-risk HPV (75%) and abnormal cytology (22%). Infection with multiple types was common. Higher-risk viruses were not over-represented in, and no dramatic decrease in HPV prevalence was observed in, older women. Cytological abnormalities were detected in only 3.74% of women who tested negative for high-risk HPV, but were found in 24.2% of high-risk HPV positive women.

Conclusion: HPV testing on self-collected tampon samples was feasible, highly sensitive and demonstrated a high negative predictive value for current cytological abnormalities in this population.

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Introduction

Cervical cancer remains the malignancy that is responsible for the highest mortality from cancer in developing countries.¹ In 1999, the South African Cancer Registry estimated that the lifetime risk of developing cervical cancer was one in 26.² Currently, there are no accurate available data on cervical cancer incidence in South Africa, but the World Health Organization estimates it to be approximately 26.6 per 100 000.³ It is widely postulated that the incidence is

probably higher, largely because many cases are still undiagnosed or unreported.³⁻⁵

Screening for cervical cancer identifies patients with precancerous lesions and offers them the opportunity of preventing the disease with effective treatment. Mortality from this cancer is very low in developed countries, owing to widely instituted population-based screening for cervical cancer, which results in prevention and early detection. To the contrary, the prevalence of and mortality from cervical cancer

remains unacceptably high in developing countries, which have not implemented screening protocols. It is expected that mortality from cervical cancer in these countries can be lowered with any screening test, provided that the necessary coverage, appropriate screening interval, as well as treatment and follow-up of screen-positive individuals can be achieved.⁶ To date, the logistical challenges of a speculum and cytology-based screening programme have prevented effective implementation in most developing environments, necessitating the urgent investigation of alternatives.

HPV-based screening offers several potential advantages over cytology-based screening, including increased sensitivity with regard to detecting precancerous and invasive lesions.^{7,8} It also has the potential to increase the screening interval and to reduce inter-observer variability in the laboratory.⁹⁻¹² As a molecular test, HPV-based screening offers the possibility of the sample being self-collected, potentially reducing human error at the point of sample collection. Self-sampling and clinician collection methods for high-risk HPV testing have been compared extensively in the literature, and are usually reported to have comparable sensitivities.¹³⁻¹⁵

The potential for self-collection offers important benefits to countries in which cultural and economic barriers may limit either the availability or acceptance of healthcare worker examinations, specialised instruments, light sources and sterilisation equipment. However, certainty about the accuracy of self-sampled HPV screening is essential before widespread implementation of the test. It is imperative to know that the results compare favourably with the current gold standard (healthcare worker-collected cervical cytology), and that patients in a specific cultural and socio-economic settings accept the method, and can reliably collect their own samples with minimal instruction.

The primary objective of the current study was to evaluate the potential role of HPV testing of self-collected vaginal samples as an alternative screening method to the currently used healthcare worker-collected cytology testing in a South African urban and peri-urban population. This population was considered to be comparable to many urbanised or partly urbanised populations in the developing world. Secondary objectives were to assess the incidence of high-risk HPV infection, and of existing cervical cytological lesions in this population of mostly unscreened women in view of limited available data from other South African populations.^{16,17}

Method

The study recruited women attending community health centres near the central business district (CBD)

and peri-urban areas of Tshwane, situated in Northern Gauteng. Samples were collected at the East Lynne Clinic (located approximately 20 km from the CBD in the east), old Pretoria Academic Hospital (within the CBD), and at the Phomolong Clinic (situated 20 km from the CBD in the northern part of Tshwane). Adult women attending for any primary health-related problem and who were eligible for cervical cancer screening were invited to participate in the study. Complete study information was provided in printed format and discussed with potential participants before written consent was obtained. The study protocol was reviewed and approved by the Research Ethics Committee of the University of Pretoria (number 210/2008).

Nursing staff underwent in-service training with regard to the method of sample collection, prior to the start of the trial. Quality control visits were undertaken by members of the research team on a regular basis. An hour before the cytology procedure, participants were given a non-applicator mini tampon and asked to insert it into the vagina. Tampons were removed after an hour, and directly placed in a phosphate-buffered saline and 10% methanol solution by the study participants. In addition, a conventional cervical cytology smear was performed for each participant by the clinic staff using a metal vaginal speculum and available wooden Ayres[®] spatulas. The tampon and cytology smear were both placed in a labelled envelope and sent to the National Health Laboratory Service at the University of Pretoria.

Specimens were transported in phosphate-buffered saline and stored at room temperature until tested. Specimen preparation consisted of three washing cycles, each with 2-ml phosphate-buffered saline, followed by spinning down and resuspension.^{15,18}

DNA extraction was performed in batches on washed cell pellets of the tampon specimens using DNA[®] Isolation Kit (Roche Molecular Systems, Branchburg, USA) on the MagNA Pure automated extraction system. HPV genotyping was performed using the Linear Array[®] HPV Genotyping Test. The pool of primers was designed to amplify HPV DNA from 15 high-risk genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82), three probable high-risk genotypes, and 19 low or undetermined-risk types. The β -globin gene was amplified concurrently to assess cellular adequacy, extraction and amplification for each individually processed specimen. Strict procedures were followed to avoid contamination. Negative and positive controls were included in each run, especially in the light of the unusual collection and transport method.

The cervical cytology slides were stained and processed using routine conventional cervical cytology methodology. The Bethesda System of classification was used to report the cytology results. Women with

cervical cytology abnormalities [atypical squamous cells, atypical glandular cells, low-grade squamous intraepithelial lesion (LSILs) and high-grade squamous intraepithelial lesion (HSILs)] were referred to Steve Biko and Kalafong Academic Hospitals for further management as per standard protocol based on cytology results. HPV results were not communicated to the healthcare teams or patients.

Statistical analysis

Epidemiological, cytology and HPV results were captured centrally and analysed, using Stata® version 11 software. High-risk HPV positivity implied positive results for any of the 15 listed types, while positivity for any of the eight HPV types most commonly found in invasive cancers (16, 18, 31, 33, 35, 45, 52, 58) was termed the “top 8”.^{19,20} Data were not stratified, and simple descriptive statistics were generally used, consisting of proportions. Unadjusted odds ratios (ORs) were used to predict the odds of negative HPV and other variables, and 95% confidence intervals (CI) determined.

Results

Seven hundred and twenty women were recruited to the study. Women who had incomplete data ($n = 89$) were excluded from further analysis. Complete data were available for 631 women. The average age of the study population was 40. The median age was also 40 years. The youngest patient was 16 years of age and the oldest 83. Women were distributed almost evenly within the four age groups (Table I).

Table I: Cytology results pertaining to the different age groups

Age group	Number	Abnormal cytology (%)
29 years of age and younger	121	22 (18.2)
30-39	175	39 (22.2)
40-49	171	18 (10.5)
50 years of age and older	141	13 (9.2)
Total group	608	92 (15.1)

Abnormal cytology was reported in 15.4% of the total study population. The abnormal cytology rate was highest (22.2%) in women aged 30-39 years of age, and declined with age over the next two older age groups. The unadjusted ORs were determined to predict the chance of each age group having abnormal cytology when compared with that in women who were younger than 30 years of age. Patients in the age group 30-39 years had an OR of 1.29 (95% CI: 0.72-2.31)

for abnormal cytology, while patients in age groups 40-49 years and 50 years and older were less likely to have cytological abnormalities than the youngest group, i.e. OR 0.53, 95% CI: 0.27-1.04, and OR 0.46, 95% CI: 0.22-0.95, respectively. These differences were not statistically significant.

The overall prevalence of high-risk HPV infection in the study group was 58% ($n = 354$). Of these positive women, 55% had evidence of infection with multiple high-risk HPV types (24% with two types, 14% with three types and 17% with four or more types). The nature and distribution of the multiple infections is shown in Figure 1.

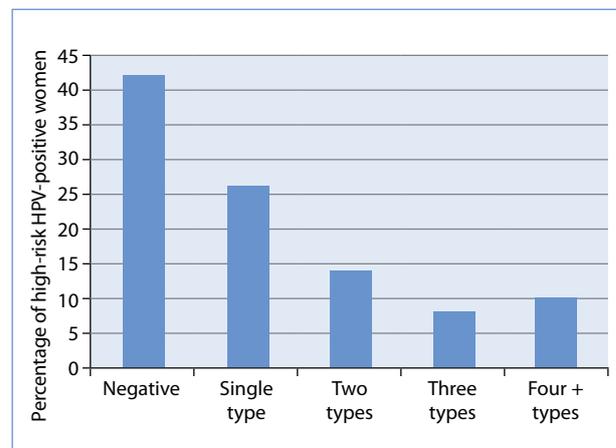


Figure 1: Percentage of women with single versus multiple high-risk human papillomavirus infections

The prevalence of high-risk HPV infection was especially high in young women, with a prevalence of 68% in women who were younger than 29 years of age. It peaked at almost 75% in women aged 30-39, and then slightly decreased in both older age groups. Of the high-risk HPV positive women, the vast majority (88%) had evidence of infection with one of the eight most oncogenic viruses. The two most common oncogenic viral types, HPV 16 and 18, were detected in 40% (143/354) of high-risk HPV-positive women. Both viruses were present in 23.5%. When compared to positivity for all HPV types (58% overall), the “top 8” viral types were present in 51% of the study participants in all age groups. The more oncogenic viral types are thought to be more persistent, but these viral types were not observed to be over-represented in older HPV-positive women. The high-risk HPV types and prevalences are summarised per age group in Table II.

The results of high-risk HPV testing and cytology were compared to evaluate the potential role of HPV-based self-screening as an alternative to conventional cervical cytology in South African women. A normal high-risk

Table II: High-risk human papillomavirus DNA results pertaining to the different age groups

Age group	High-risk HPV positivity n = 354, (%)	The HPV "top 8" ^{**} n = 314	HPV 16 n = 94	HPV 18 n = 65	HPV 16 and HPV 18 ^{**} n = 143
29 years of age and younger	83 (68.6)	75 (62)	25 (20.7)	13 (10.7)	33 (27.3)
30-39	131 (74.9)	117 (66.9)	36 (20.6)	23 (13.1)	54 (30.9)
40-49	76 (44.4)	64 (37.4)	15 (8.8)	13 (7.6)	25 (14.6)
50 years of age and older	64 (45.4)	58 (41.1)	18 (12.8))	16 (11.3)	31 (22)
Total study group (%)	354 (58.2)	314 (51.6)	94 (15.5)	65 (10.7)	143 (23.5)

HPV: human papillomavirus

*: The HPV "top 8" refers to positivity for any of the eight most common oncogenic types, namely human papillomavirus 16, 18, 31, 33, 35, 45, 52 and 58

**.: HPV 16/18 refers to positivity, either for HPV 16 or HPV 18, or both types

Table III: Correlation of the results of the high-risk human papillomavirus and cytology tests

HPV status	Cytology (normal)	Cytology (abnormal)	Cytology (total)	Predictive values of high-risk HPV test	OR for abnormal cytology, (95% CI)
High-risk HPV (negative)	257	10	267	NPV 96.25%	0.12 (0.06-0.24)
High-risk HPV (positive)	270	86	356	PPV 24.16%	1

CI: confidence interval, HPV: human papillomavirus, NPV: negative predictive value, OR: odds ratio, PPV: positive predictive value

HPV had a negative predictive value of 96.25% in envisaging the presence of normal cervical cytology findings on conventional cytology smears. Cytological abnormalities were detected in 3.74% (10/267) women who tested negative for high-risk HPV, while cytological abnormalities were identified in 24.2% of high-risk HPV-positive women. Abnormal cytology results for women who were high-risk HPV-negative were: HSILs in two women (0.75%), LSILs in two women (0.75%) and atypical cells of undetermined significance in six women (2.25%). No histology was available to confirm the diagnosis. Unadjusted ORs that predicted abnormal cytology in the different age groups of the women with high-risk HPV are shown in Table III. Women who tested negative for high-risk HPV were far less likely to have abnormal cytology than women who tested positive for high-risk HPV. This difference was found to be statistically significant (OR 0.12, 95% CI: 0.06-0.24).

Discussion

In this study, tampon sampling was chosen, using phosphate-buffered saline as a transport medium. Previous studies conducted in this unit and others have demonstrated high levels of acceptance by urban patients and a high yield of DNA, including cervical material, after 1-2 hours of exposure. In addition, these sampling and transport mediums are freely available

and affordable. In the current study, the DNA yield was not determined, but only three samples were invalid.

The prevalence of abnormal conventional cervical cytology in this population was 15.4%. This figure is consistent with the findings of Richter, Becker, Horton and Dreyer⁶ who reported abnormal cytology in 17.2% of unselected women tested in Gauteng province. A study from the Western Cape reported a prevalence of abnormal cytology that ranged from 7-10%.¹⁶ The high prevalence (especially in surveys from the northern part of South Africa) draws serious and urgent attention to the unchecked epidemic of cervical pre-neoplasia and cancer in South Africa.

Cervical dysplasia and abnormal cytology is well known to be caused by persistent high-risk HPV infection. An equally high prevalence of high-risk HPV infection was found in this study population. The prevalence of cervical high-risk HPV DNA positivity was 57%, which is surprisingly high for a population-based study. In addition, many HPV-positive women tested positive for multiple HPV types.

Both abnormal cytology rates and HPV infection rates were highest in the age group of 30-39 years. HPV prevalence in the general population is usually reported to peak in women who are younger than 25 years of

age, and this falls rapidly in women aged 30-40.¹⁷ This trend was clearly not present in this study population, and was also reported by Richter, Becker, Horton and Dreyer.⁶ Multiple factors may be responsible for why the trend is for HPV infections to persist, rather than to clear. These may include poor herd immunity against HPV, partly because of high HIV infection rates and sexual behaviour patterns. The age distribution of both HPV prevalence and cytological abnormalities raises the all-important question of whether or not age groups should be included in a screening policy for South African women.

HPV types 16 and 18 were among the most frequent types, which is in keeping with the literature published on HPV prevalence.¹⁸ As demonstrated in Table II, testing only for HPV types belonging to the "top 8" category (consistent with the types previously described by De Sanjose et al²⁰ and confirmed by our own data),¹⁹ rather than for all 15 high-risk HPV types, did not significantly lower the number of women who tested positive. This finding limits the use of the "top 8" group of viruses to increase the specificity of viral screening, while retaining high sensitivity. Primary HPV testing, as a screening method using HPV 16 and 18 only, will detect or predict approximately 60% of invasive cancers. The "top 8" cause 85% of invasive carcinoma. Screening for the "top 8" will thus prevent 85%. It was demonstrated in this study that the majority of patients who were HPV- (non-16 and non-18) positive, were infected with the "top 8" oncogenic viruses (314/354). However, this test will be positive for 58% in a pooled high-risk HPV analysis.

The antenatal HIV survey of 2007-2010 showed that the prevalence of HIV is increasing in pregnant women aged 30 years and older.²¹ This is also the age group which demonstrated the highest prevalence of high-risk HPV and cytological abnormalities in this study. HIV infection has been shown to greatly contribute to cervical neoplasia and persistent HPV disease. In addition, it may partly explain the high prevalence of dysplasia and infection with multiple HPV types, as observed in the current study. It is also probable that the long-term unscreened and untreated status of this population contributed to a changed epidemiology, with increasingly prevalent HPV infection in the community, and increased shedding of viral copies.

In this study, the prevalence of abnormal cytology in women who were negative for high-risk HPV on self-collected samples was 3.74%. 96.3% had normal cytology. This finding is in keeping with other estimates of the negative predictive value of HPV tests that ranged from 96-100%.²² The study design was limited by the absence of histology data for these "false negatives".

The cross-sectional design of this study limited calculations of the value of HPV when screening

for cervical dysplasia and when detecting current lesions, and cannot confirm the predictive value for future disease. Long-term follow-up of patients who were HPV-positive in other trials confirmed a greatly increased risk of developing cervical dysplasia than that in HPV-negative women. In addition, it is well known that a single round of cytology testing underestimates disease and that many women who test positive for high-HPV will already harbour undiagnosed dysplasia. When the future development of lesions and underdiagnosis of existing disease is taken into account, the positive predictive value of HPV testing is considerably improved. This study did not address the problem posed by the relatively low specificity of HPV screening, which necessitates creative methods to triage, treat or follow-up large numbers of women with positive high-risk HPV tests.

Conclusion

The prevalence of high-risk HPV and abnormal cytology in this study population was very high. These findings emphasise the need to implement effective screening and a comprehensive cervical cancer prevention strategy. HPV vaccination should be on top of the priority list, followed by an effective screening programme.

In this study, a negative high-risk HPV test on self-collected samples accurately predicted normal cytology and was at least as sensitive as a cytology test. Although the study was not designed to evaluate participants' perceptions of tampon testing, we can report effortless acceptance, understanding and almost universal uptake of this sampling method. This finding is of particular importance in this largely unscreened population, where poor access to and low acceptability of a gynaecological examination may hinder healthcare worker-collected cervical cytology screening. Self-screening is expected to improve coverage in many populations in which cytology-based screening has not been effectively implemented.

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