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Research Article

A comparative analysis of conventional Pap smear cytology, liquid based cytology and colposcopy clinical impression with colposcopy biopsy histology as gold standard in women undergoing colposcopy in Kenyatta National Hospital

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ABSTRACT

Background: Cervical cancer is one of the most common female malignancies worldwide. Since the introduction of conventional Papanicolaou smear mortality from cervical cancer has reduced considerably. Despite its success, it has sensitivity of only 51% and false negative rate of 5-10%. Approved liquid based cytology (LBC) products by FDA claim a 65-percent increased detection rate of high grade squamous intraepithelial lesions (HGSIL) compared with conventional smears, as well as decreased unsatisfactory sample rates. Evidence shows that liquid based preparation is more sensitive and accurate for the detection of both squamous and glandular lesions of the cervix. Studies of the accuracy of liquid based preparations reports sensitivity of 61-66% and specificity of 82-91%. Objective of current study is to compare the performance of conventional Pap smear cytology, liquid based cytology and colposcopy clinical impression with colposcopic biopsy as the gold standard among women eligible for colposcopy in Kenyatta National Hospital, Kenya.

Methods: This was a hospital-based comparative cross-sectional study. Convenient sampling over a period of 4 months was used to recruit clients referred to colposcopy clinic with abnormal Pap smear results.

Results: A total of 73 patients referred with abnormal pap smears were recruited into the study. The mean age of the patients was 38 yrs (SD ±10). About 45% of the patients interviewed did not have knowledge of Pap smear testing. Both the results of referral Pap smear and repeat Pap smear were predominantly low grade squamous intraepithelial lesions (LGSIL) or HGSIL. With biopsy results being the gold standard**,** Liquid based cytology had a higher specificity of 75% when compared with conventional pap smears' 11%.

Conclusions: Even though colposcopy clinical impression has the highest agreement with colposcopy biopsy it's a diagnostic and not a screening test, hence Liquid based cytology showed better performance as a screening test compared to conventional Pap smear. In general, there was good agreement for cytological results of repeat CPAP and LBC. We therefore recommend that for patients referred with abnormal pap smears requiring a repeat pap smear, liquid based cytology is used due to its higher specificity compared to conventional Pap smear.

Keywords: Cervical, Cancer, Screening, Pap smear, Liquid-based cytology, Colposcopy biopsy histology, Sensitivity, Specificity, Predictive value

INTRODUCTION

Cervical cancer whose aetiology is oncogenic human papilloma virus (HPV) is the second most common malignancy among women worldwide after breast cancer, but is the most common among women in developing countries.[1,2] Globally, cervical cancer constitutes about 12% of all cancers in women.[1,2] In 2002, in developing countries, 493,243 new cases were observed and 273,606 deaths, corresponding to a 55 percent mortality rate.[2] Eighty-three percent of all cases of cervical cancer worldwide occur in developing countries; this results in a cumulative risk of 1.5 percent for developing cervical cancer by the age of 65 years. Further, while there has been a 75 percent decrease in the incidence and mortality of cervical cancer over the past 50 years in developed countries the same has not been realized in the sub-Saharan African region[2]. This discrepancy is largely due to the widespread institution of cervical cancer prevention programs in developed countries, which are essentially non-existent in many developing countries. Based on a recent meta-analysis of process of care failures in the prevention of cervical cancer, poor screening history was the primary factor: 54 percent of invasive cervical cancer patients had inadequate screening histories and 42 percent were never screened.[3]

In Kenya, the true incidence of cervical cancer is not known, but it is the most common gynecological malignancy. It is estimated to be 2,454 women per year with annual number of deaths estimated at 1,676 women. In the absence of accelerated interventions for screening, detection and early treatment, the incidence of cervical cancer is projected to rise to 4,261 resulting in 2955 deaths in 2025.[4] HIV-infected women, compared to their HIV-uninfected counterparts have an increased risk for infection with carcinogenic ‘‘high risk’’ types of HPV.[5] In addition, HIV-infected women also, are frequently infected with multiple HPV types, and have a higher chance of presenting with a persistent infection or progression of these lesions to invasive cancer.[6]

The conventional Pap smear is the standard screening test for cervical neoplasia in Kenya. Despite its success, the Pap smear has high false-negative rates due to poor sensitivity (51%; 95% confidence interval [CI], 0.37–0.66). The false positive rate of the Pap smear is thought to be in the range of 1% to 10%.[7] False-positive test results lead to needless follow-up procedures. At a minimum, the patient will probably be subjected to more screening (thereby increasing the risk of still more false-positive results). The workups for an abnormal Pap smear typically include colposcopy and biopsy. Colposcopy biopsy is considered gold standard in the diagnosis of pre-malignant lesions.

This study sought to determine and compare the agreement, sensitivity, specificity and predictive values of conventional Pap smear cytology, liquid based cytology and colposcopy clinical impression with colposcopic biopsy as the gold standard among women eligible for colposcopy in Kenyatta National Hospital, Kenya.

METHODS

Study design

This was a hospital-based comparative cross-sectional study.

Study site and setting

The study was conducted at Kenyatta National Hospital’s (KNH) colposcopy clinic. KNH is main national referral and teaching hospital in Kenya. It is the teaching hospital for the College of Health Sciences of the University of Nairobi and for the Kenya Medical Training College, Nairobi.

The colposcopy clinic is conducted every Thursday and Friday by a consultant gynaecologist competent and skilled to perform colposcopy. On average a total of 110 patients per month undergo management in this clinic. Patients eligible for Colposcopy are those with abnormal Pap smear cytology results, which include: persistent low grade squamous intraepithelial lesion (LGSIL), LGSIL, abnormal squamous cells of unknown significance (ASCUS), and high grade squamous intraepithelial lesion (HGSIL). A confirmation of cervical intraepithelial neoplasia $\geq$ CIN 2/3 without invasion, after colposcopy biopsy histology, are treated with loop electrosurgical excision procedure (LEEP). Patient whose histology confirms invasive cancer are triaged to the invasive cancer protocol[8].

Study population

Women aged more than 18 years of age, referred to colposcopy clinic with abnormal Pap smear cytology and gave written informed consent. Women were ineligible to participate if they had previously been treated for a cervical abnormality and if they were pregnant.

Sample size and sampling procedure

The false positive rate of the Pap smear is thought to be in the range of 1% to 10%. Taking the average 5% as the estimated rate of false positives and a precision of ±5% and a 95% confidence interval, we estimated we would require a sample of 73 patients. Eligible clients were consecutively recruited into the study over a period of 4 months between August and November 2011 until the desired sample size was attained.

Study related procedures

After informed consent, patients eligible for colposcopy had a Pap smear collected by spilt technique, to avoid double collection of samples. Pap smear specimen was

collected by a cytobrush some smeared on a slide (conventional Pap smear cytology [CPAP]) and the reminder collected in a vial containing preservative for liquid based preparation processing (Liquid based Cytology [LBC]). After the Pap smear is collected colposcopy procedure was carried out with a colposcopy clinical impression and biopsy. The Pap smear cytology and colposcopy biopsy histology were read at the Department of Human pathology University of Nairobi. Random numbers of all samples were re-read, every 5th CPAP and LBC sample by a 2nd pathologist. Pap smear cytology results were availed to patient files in 4 weeks, while colposcopy biopsy histology results after 6 weeks.

Data collection

Structured questionnaires were used to collect data at the time of recruitment while cytology and histology results were collected from patients files.

Data variables

Table 1: Data variables.

Merged classification	Reported classification
Normal	Normal
Pre-cancerous	Atypical glandular cells of unknown significance (AGUS)
	Atypical squamous cells of undetermined significance (ASCUS)
	Low-grade squamous intraepithelial lesions (LSILs)
Cancer in situ	Carcinoma in-situ (CIS)
	High-grade squamous intraepithelial lesions (HSILs)
Cancer	Squamous cell carcinoma
	Adenomacarcinoma
Unspecified	Inflammatory cells/unsatisfactory

Based on the clinical management indicate for the various reported classifications, test results were re-classified to take this into account as shown in table above. For the sensitivity and specificity testing all other classifications that were not indicated as normal were coded abnormal for each of the diagnostic tests.

Data analysis and presentation of results

Data entry was done in Microsoft Access and in-built validation checks were instituted. Data analysis was performed using Statistical Package for Social Scientists (SPSS Version 19.0). Nominal variables were summarized using frequencies and percentages whereas continuous variables were summarized using measures of central tendency (mean median, minimum, maximum and standard deviation). Tests for statistical association were done using Chi squared tests and Fishers Exact tests nominal variables and analysis of variance for continuous variables. Sensitivity analysis was carried out to determine sensitivity, specificity, positive predictive value, negative predictive value were estimated for each of the tests.

Ethical considerations

Approval was sought from the Ethics Committee of Kenyatta National Hospital and University of Nairobi Ethical Review Committee. Informed consent was sought from all participants. Patient confidentiality was maintained and only de-indentified data was analysed.

RESULTS

A total of 73 women who were referred for cervical cancer screening were enrolled into the study. Their ages ranged from 18 – 60 years, with a mean age (SD) of 38 (± 10) years. Literacy levels were high in this group with only 7% (5) having no education while 11% (8) had tertiary education. Majority (60%) of the women were married and in a monogamous relationship while 18% (13) were ever married (divorced, separated, and widowed).

Table 2: Socio-demographic and reproductive characteristics of respondents of women undergoing colposcopy in Kenyatta national hospital, 2011.

		Number	Percentage
Age	Mean (SD)	38 [10]	
Marital status	Single	10	14
	Monogamous	44	60
	Polygamous	6	8
	Separated	7	10
	Divorced	1	1
	Widow	5	7
Education level	None	5	7
	Primary	28	38
	Secondary	32	44
	Tertiary	8	11
Age at first intercourse	<15 years	11	15
	15-20 years	49	68
	20-25 years	10	14
	25-30 years	2	3
No. of recent sexual partners	<2	63	86
	2 - 5	10	14
Age menarche	Mean (SD)	16 [2]	
Parity			
Parity	Median (IQR)	2 [1-3]	
Pap smear result at entry	Normal	0	0
	Pre-cancerous	30	41
	Cancer *in situ*	40	55
	Cancer	3	4

More than 80% of the women experienced their first sexual debut before the age of 20 of which for 15% (11) was before age 15 years while 68% (49) was in the age group 15 – 20 years. The mean age (SD) of menarche was 16 (2) years. Further their parity ranged 0 -5 with a median (IQR) of 2 (1- 3). The sexual histories on the number of recent sexual partners showed that majority 86% (63) had less than two partners while only 1 had more than 5 partners in the recent past. Socio-demographic and reproductive characteristics are described in detail in Table 2.

Table 3 shows the distribution of results from the various tests (CPAP, LBC and colposcopy clinical impression) compared to gold standard (biopsy) results. Further the agreement of the test results to those of biopsy results is reported in Table 4 below. Overall there was about the same agreement rates of 73% between repeat conventional Pap smear and Liquid based cytology when compared to colposcopy biopsy test results. However 82% agreement rate between colposcopy impression findings and biopsy results was identified.

Table 3: *Comparison of conventional Pap smear cytology, liquid based cytology and colposcopy clinical impression with colposcopy biopsy histology as gold standard in women undergoing colposcopy in Kenyatta national hospital, 2011.

	Colposcopy biopsy histology					Total
	Unspecific	Normal	Pre-cancerous	Carcinoma *in situ*	Cancer	
Conventional Pap smear cytology						
Normal	8	4	13	5	2	32
Pre-cancerous	1	0	3	6	0	10
Cancer *in situ*	1	3	3	11	6	24
Cancer	0	0	0	0	0	0
Unspecific	1	1	2	3	0	7
Liquid based cytology						
Normal	1	1	2	2	0	6
Pre-cancerous	5	1	8	7	1	22
Cancer *in situ*	2	4	6	11	7	30
Cancer	0	0	0	1	0	1
Unspecific	3	2	5	4	0	14
Colposcopy clinical impression						
Normal	1	4	3	2	0	10
Pre-cancerous	7	1	12	6	1	27
Cancer *in situ*	2	3	5	17	3	30
Cancer	0	0	0	0	4	4
Unspecific	1	0	1	0	0	2
Total	11	8	21	25	8	73

*the conventional Pap smear, liquid based cytology and colposcopy were all done in the same day

Table 4: *Agreement between conventional Pap smear cytology, liquid based cytology and colposcopy clinical impression with colposcopy biopsy histology as gold standard in women undergoing colposcopy in Kenyatta national hospital, 2011.

	Agreement	Expected agreement	Kappa	Standard error	Z	p-value
Conventional Pap smear cytology	73.63%	66.82%	0.201	0.073	0.83	0.002
Liquid based cytology	72.95%	67.77%	0.161	0.079	2.03	0.021
Colposcopy clinical impression	81.85%	71.05%	0.373	0.072	5.21	< 0.001

*the conventional Pap smear, liquid based cytology and colposcopy were all done in the same day

Table 5: *Sensitivity, specificity and negative predictive value of conventional Pap smear cytology, liquid based cytology and colposcopy clinical impression with colposcopy biopsy histology as gold standard in women undergoing colposcopy in Kenyatta national hospital, 2011.

	Sensitivity % (95% CI)	Specificity % (95% CI)	Positive predictive value % (95% CI)	Negative predictive value % (95% CI)
Conventional Pap Smear cytology	50 (39 – 61)	57 (46-68)	13 (5 -20)	90 (83 - 97)
Liquid based cytology	13 (5 -20)	92 (86 - 98)	17 (8 – 25)	90 (83 - 97)
Colposcopy clinical impression	50 (39 – 61)	91 (84 - 97)	40 (29 - 51)	94 (88-99)
*the conventional Pap smear, liquid based cytology and colposcopy were all done in the same day				

Repeat CPAP and colposcopy diagnostic tests had the same sensitivity of 50%. Colposcopy performed better than the other two screening tests demonstrating a positive predictive value and a negative predictive value of 40% and 94% respectively. However compared to Repeat CPAP, LBC had better specificity of 91% hence being a better test despite the two tests having the same negative predictive value of 90%. Other properties of the screening test compared to biopsy results are presented in Table 5.

DISCUSSION

Cervical cancer continues to be one of the leading reproductive cancers especially in the developing world, evidenced by prevalence of 45% (women with either HGSIL/CIS/SCC/Adenocarcinoma) in this cohort, a result consistent with other studies undertaken in Nigeria[9] and other developing countries.[10] Further the mean age of the patients was 38 yrs (SD ±10). More than 75% was below 44years with most with peak in the age range of 40-44 years. These findings are similar to those of Oguntayo O et al. who in a retrospective study done in Nigeria found the mean age of CIN to be 37.6 years with a combined prevalence of 48/1000. Highlighting that cervical cancer affects women in their prime economic productive age.[9,11]

Data from this study demonstrate that, colposcopy clinical impression has the highest agreement of 82% with colposcopy biopsy histology. Conventional Pap smear and liquid based cytology had similar agreement of about 73%. Conventional Pap smear cytology and colposcopy clinical impression has the same sensitivity of 50% with liquid based cytology having the lowest (13%). Liquid based cytology and colposcopy clinical impression have a very high specificity and negative predictive values of over 90%. The specificity and sensitivity of conventional Pap smear cytology and liquid based cytology compares well with findings of other studies.[12-14]

For cancer screening and diagnosis especially in resource limited settings where screening is opportunistic, one needs a test that has a high specificity and negative predictive value, and that can guarantee a negative screen or diagnosis for cancer of the cervix. From our results, liquid based cytology and colposcopy clinical impression would be the best way to screen and make a diagnosis of cervical cancer. The limitation of these two tests however would be the risk of overtreatment. In our setting, over-treatment is accepted because there is a higher risk of disease progression and lost-to-follow-up coupled with late presentation in advanced disease.[8]

The limitation of this study was that, this study showed increased number of unsatisfactory smears in liquid based slides contrary to other studies done 19% against 6% for conventional pap smears. This result has been noted in other split-sample studies 33; the first portion of the sample was used to make the conventional smear and may have contained most of the endocervical component. Another factor may have been the necessity of introducing the 'broom' sampling device for this study. The spatula endocervical brush combination might have yielded a higher proportion of samples with endocervical component. Liquid based cytology showed better performance as a screening test for cervical cancer than conventional Pap smear. A second challenge was the lack of a quality assurance process in the reading of the results; however this could be attributed to the routine environment in which this study was undertaken. Despite the above challenges we feel that the following strengths should be taken into account. Firstly, the use of split technique and collection of all samples in one time point allowed for comparison of the performance tests for the same disease status while cutting down costs for repeated sample collection during different time periods. Secondly, study procedures were undertaken in routine settings and hence promoting the generalizability of these results to other low resource settings.

CONCLUSIONS

Cervical cancer screening is quickly becoming an urgent public health problem which needs immediate attention from all concerned parties. One of the immediate strategies should be sustained health education and

awareness creation to promote early detection of cases through appropriate screening strategies. These results indicate that the three screening tests performed well and are viable alternatives for consideration as screening options in different settings based on the availability of resources and technical capacity. Even though colposcopy clinical impression has the highest agreement with colposcopy biopsy it's a diagnostic and not a screening test, hence Liquid based cytology showed better performance as a screening test compared to conventional Pap smear. In general, there was good agreement for cytological results of repeat CPAP and LBC. We therefore suggest that for patients referred with abnormal pap smears requiring a repeat pap smear, liquid based cytology is used due to its higher specificity compared to conventional Pap smear where matters of cost are not an issue. However, due to the high cost of this new technology, in the normal population, conventional Pap smear remains the screening test of choice.

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