PAP SMEAR CYTOLOGICAL OUTCOMES IN HIV POSITIVE WOMEN REFERRED FOR VISUAL INSPECTION TESTS IN MURANG'A DISTRICT HOSPITAL, MURANG'A COUNTY, KENYA

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ABSTRACT

Background: Cervical cancers are some of the leading causes of deaths among women of reproductive age in the developing world and are commonly either diagnosed late or misdiagnosed. Abnormal cervical cytological results in HIV-positive women are much higher than what is found in the general population. Though Papanicolaou (Pap) smear is the gold standard in screening for cervical cancers, the method is not being used widely in the resource constrained countries. The methods that are currently being used are visual inspection with acetic acid (VIA) and visual inspection with Lugol's iodine (VILI

Broad objective: The objective of the study was to investigate Pap smear cytological outcomes in HIV positive women referred for visual inspection tests in Murang'a District Hospital.

Methods:in this comparative cross sectional study, 78 HIV positive women attending the reproductive health section of the CCC aged between 18 and 50 years were subjected to Pap smear VIA and VILI after consenting.

Results: The results showed majority of the HIV positive women (about 49%) were in monogamous marriage and about 24% were single and never married. The rest were either separated or widowed. Most respondents had less than 2 recent sexual partners. The positivity of Pap smear in this study was 11.5 %. Socio demographic characteristics and Pap smear results were not correlated in the study population. The positivity of both VIA and VILI in this study was 3.8 %. There was 100% concordance in results for VIA and VILI. There were more abnormalities detected on Pap smear than on VIA/VILI (11.5% vs. 3.8%). The degree of agreement between Pap smear and VIA/VILI was weak, with a kappa (k) of -0.061.

Conclusion: There were no respondents found to be in polygamous marriage. Pap smear detected more abnormalities than VIA/VILI. VIA/VILIshouldn't replace Pap smear as the primary screening tool for universal screening in Murang'a District Hospital.

Index terms – Cervical Cancer; Murang'a District Hospital; Pap smear; VIA; VILI.

INTRODUCTION

The early detection and accurate diagnosis of cervical cancer is the key to the determination of its prevention, management and clinical outcomes. Nearly all cases of cervical cancer are caused by human papillomavirus (HPV), and only two high risk HPV types, 16 and 18, are responsible for about 70 percent of all cases of cervical cancers reported in the world (Watson, 2009). Incidences and mortalities related to cancer of the cervix are both decreasing in developed countries because of effective cytological analysis screening programs through Papanicolaou (Pap) smear (Haydaroglu, 2004). This is not possible in the developing countries because Pap smear screening is expensive; there is shortage of trained cytopathologists and also due to the fact that the test is accomplished in several stages (ACCP, 2002). These potential difficulties in cytology-based programs have led Kenyan health authorities to consider using other low-cost screening technologies like visual inspection with acetic acid (VIA) and Lugol's iodine (VILI). These tests are however associated with false positives in non-cancerous lesions of the cervix hence predisposing these women into expensive cytotoxic chemotherapies. In cases of false negatives they can fail to detect cervical abnormalities leading to poor management of

disease. This study therefore aimed at generating data on the comparative effectiveness of VIA/VILI and Pap smear tests to establish the degree of agreement on the results between the two in the diagnosis of cervical cancer among human immunodeficiency virus (HIV) positive women attending Comprehensive Care Clinic in Murang'a district hospital since this data is generally lacking

MATERIALS AND METHODS

This was a comparative cross sectional study carried out in the reproductive health section of the Comprehensive Care Centre (CCC) in Murang'a district hospital, the main referral hospital for the entire Murang'a County and its environs, Murang'a County, Kenya. A total of 78 HIV positive women aged between 18 and 50 years were recruited conveniently from the reproductive health section of the CCC. HIV infected women who declined to participate, were experiencing menstrual flow or had had hysterectomy were excluded from the study.

Ethical approval of the study protocol was obtained from Mount Kenya University Ethical Review Committee, NACOSTI and also from Murang'a district hospital. After taking an informed consent from all those within the eligibility criteria, cellular materials from the uterine cervix were collected using cytobrushes and spatulas. The materials were transferred onto glass microscope slides. The slides were fixed immediately in 70% alcohol. This was followed by VIA and VILI tests. The fixed slides were stained by Pap stain and examined microscopically in the Laboratory.

RESULTS

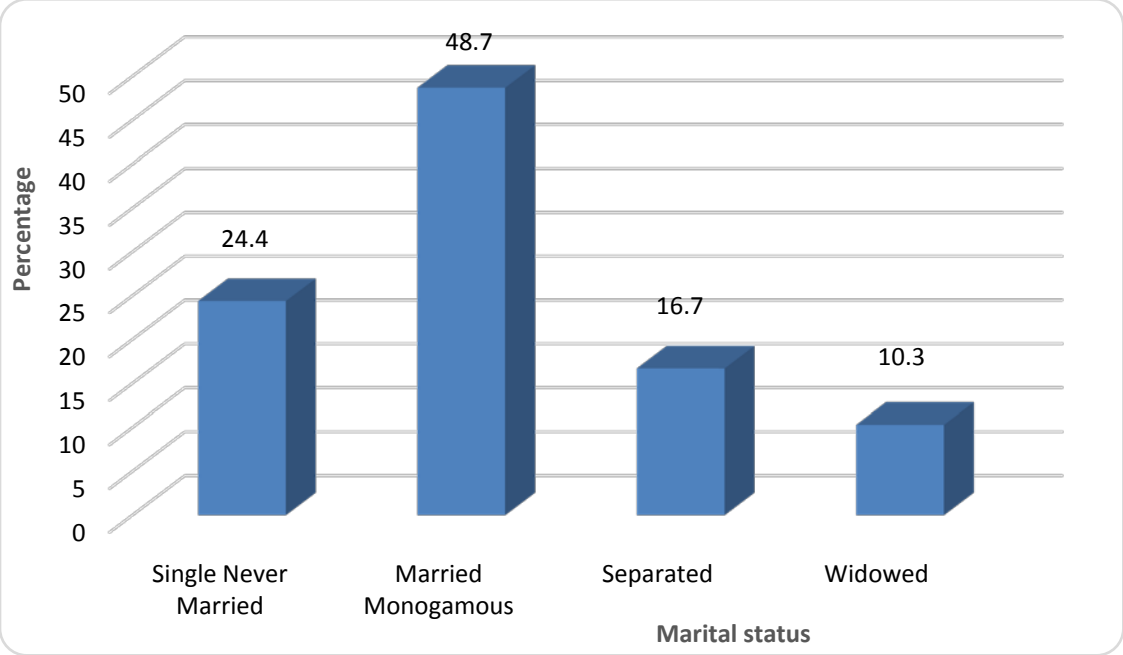

Figure 1

The study revealed that majority of the HIV positive women (about 49%) were married monogamous and about 24% were single never married. About 27% were either separated or widowed (Figure 1).

Table 1: Measures of central tendencies for age, number of children and age at menarche

Variable	N	Range	Minimum	Maximum	Mean		Std. Deviation	Variance
					Statistic	Std. Error		

Age in years	78	30	20	50	37.67	0.852	7.521	56.563
# of children	78	6	0	6	2.64	0.146	1.289	1.662
Menarche	78	8	12	20	14.56	0.17	1.5	2.249

Table 1 shows that the mean age of the respondents was 37.67 years (SD ±7.521) and this compared favorably with the modal age of 45 years and median age of 38 years. This showed the study population was normally distributed. The mean number of children was about 3 while the modalnumber of children was 2. In addition, the mean age of menarche was 14.56 years (SD ±1.5). Modal age of menarche was 15 years and the median age is about 14 years.

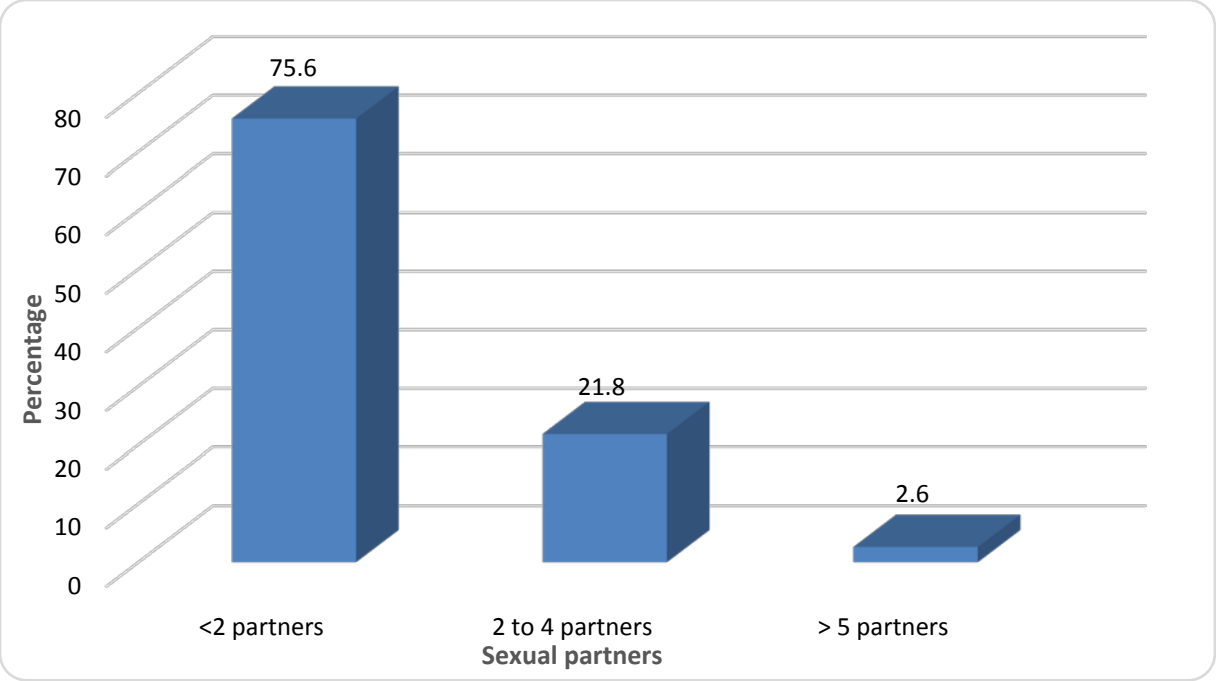

Figure 2: Recent sexual partners

The study further revealed that most respondents (75.6%) had less than 2 recent sexual partners, while 21.8% of the respondents had between 2 to 4 recent sexual partners (Figure 2).

The Pap smear results reveal 11.5% positivity (Figure 2). This means that 11.5% (95% CI: 4.42% to 18.58%) of the respondents had a positive cervical cancer screening Pap smear results.

Of the positive cervical cancer screening Pap smear results about 67% were Low-grade squamous intraepithelial lesion (LSIL) while 33% were High-grade squamous intraepithelial lesion (HSIL).

The results demonstrate that respondents' age in years and Pap smear results are not correlated in the study population. There was no statistical association between the number of recent sexual partners and the Pap smear results

Table 2: Correlations between age in years and Pap smear results

		Age in years	Pap smear results
Age in years	Pearson Correlation	1	0.005
	Sig. (2-tailed)		0.963
	N	78	78

Pap smear results	Pearson Correlation	0.005	1
	Sig. (2-tailed)	0.963	
	N	78	78

Pearson's product moment correlation coefficient shows a low positive linear relationship between the age in years and the Pap smear results (r = 0.005) that is not significantly different from Zero (p=0.963) (Table 2). The results demonstrate that respondents' age in years and Pap smear results are not correlated in the study population.

Table 3: Bivariable analysis between recent sexual partners and Pap smear results

		Pap Smear Results		χ^2	p-value
		Negative	Positive		
Number of recent sexual partners	<2 years	86.4% (51/59)	13.6% (8/59)	0.969	0.325
	>2 years	94.7% (18/19)	5.3% (1/19)		
		88.5% (69/78)	11.5% (9/78)		

Chi-square test for independence is used to test significance difference between the number of recent sexual partners and the Pap smear results. It revealed that there is no statistical association between the number of recent sexual partners and the Pap smear results (Table 3).

The VIA results reveal 3.8% positivity. This means that 3.8% of the respondents had a positive cervical cancer screening VIA results

The VILI results reveal 3.8% positivity. This means that 3.8% of the respondents had a positive cervical cancer screening VILI results

Pap smear test was taken as the gold standard for cervical cancer screening.

Table 4: VIA cervical screening tests

		Pap Smear Results		
		Negative	Positive	
VIA Results	Negative	66	9	75
	Positive	3	0	3
		69	9	78

Sensitivity = (0/9 * 100%) = 0.0%

Specificity = (66/69 * 100%) = 95.7%

This means the VIA screening test was very good at picking out the women who did not have cervical cancer (see specificity) but very poor at picking out women with cervical cancer (see sensitivity).

PPV = (0/3 * 100%) = 0.0%

NPV = (66/75 *100%) = 88.0%

This means that probability that subjects with a positive screening test truly have the disease is very low at 0.0% (see PPV) using the VIA screening test. The probability that subjects with a negative screening test truly don't have the disease is high at 88.0% (see NPV) using the VIA screening test.

Table 5: VILI cervical screening tests

		Pap Smear Results		
		Negative	Positive	
VILI Results	Negative	66	9	75
	Positive	3	0	3
		69	9	78

Sensitivity = (0/9 * 100%) = 0.0%

Specificity = (66/69 * 100%) = 95.7%

This means the VILI screening test was very good at picking out the women who did not have cervical cancer (see specificity) but very poor at picking out women with cervical cancer (see sensitivity).

PPV = (0/3 * 100%) = 0.0%

NPV = (66/75 *100%) = 88.0%

This means that probability that subjects with a positive screening test truly have the disease is very low at 0.0% (see PPV) using the VILI screening test. The probability that subjects with a negative screening test truly don't have the disease is high at 88.0% (see NPV) using the VILI screening test.

Table 6: Cross-tabulation between Pap smear and VIA results

		Pap Smear Results		
		Negative	Positive	
VIA Results	Negative	66	9	75
	Positive	3	0	3
		69	9	78

Of the 78 HIV positive women evaluated, 66 HIV positive women evaluated had negative cervical cancer test as agreed by both Pap smear and VIA results. In addition, both tests agreed that there were no HIV positive women had cervical cancer.

Table 7: Symmetric measures

		Value	Asymp. Std. Error	Approx. T	Approx. Sig.
Measure of Agreement	Kappa	-0.061	0.028	-0.638	0.524
N of Valid Cases		78			

In the symmetric table, k = -0.061, p=0.524 (Table 7). The observed level of agreement between the two tests was less than what would have been expected by chance and therefore the inter-rater reliability was unsatisfactory.

Table 8: Cross-tabulation between Pap smear and VILI results

		Pap Smear Results		
		Negative	Positive	
VILI Results	Negative	66	9	75
	Positive	3	0	3
		69	9	78

Of the 78 HIV positive women evaluated, 66 HIV positive women evaluated had negative cervical cancer test as agreed by both Pap smear and VIA results. In addition, both tests agreed that there were no HIV positive women had cervical cancer.

Table 9: Symmetric Measures

		Value	Asymp. Std. Error	Approx. T	Approx. Sig.
Measure of Agreement	Kappa	-0.061	0.028	-0.638	0.524
N of Valid Cases		78			

In the symmetric table, k = -0.061, p=0.524 (Table 9). The observed level of agreement between the two tests was less than what would have been expected by chance and therefore the inter-rater reliability was unsatisfactory.

Table 10: Cross-tabulation between VILI and VIA results

		VILI Results		
		Negative	Positive	
VIA Results	Negative	75	0	75
	Positive	0	3	3
		75	3	78

Of the 78 HIV positive women evaluated, 75 HIV positive women evaluated had negative cervical cancer test as agreed by both VIA and VILI results. In addition, both tests agreed that there were 3 HIV positive women who had positive cervical cancer test results

Table11: Symmetric measures

		Value	Asymp. Std. Error	Approx. T	Approx. Sig.
Measure of Agreement	Kappa	1	0	8.832	0
N of Valid Cases		78			

In the symmetric table, k = 1, p=0.000 (Table 11). The observed level of agreement between the two tests was excellent and therefore the inter-rater reliability was satisfactory.

DISCUSSION

Numerous studies have demonstrated that social and demographic characteristics influence the occurrence of cervical cancer among HIV positive women. The relative risk of invasive cancer is increased with increased number of sexual partners, younger age at first sexual intercourse, increasing parity, younger age at first full term pregnancy and increased duration of oral contraceptive use (Berrington, 2007). The study revealed that majority of the HIV positive women (about 49%) were in monogamous marriage and about 24% were single and never married. The rest were either separated or widowed. This contrasted with a study done in Kenyatta National Hospital by ChegeMacharia (Chege, 2013) which showed 60 % of women to be in monogamous marriages, 18 % were either separated or widowed while 14 % were single. This difference could be attributed to the choice of study site that is predominantly urban and is the national referral hospital. This particular study was done in a rural set up while the latter was in the capital city.

About 78% of the respondents had 3 or fewer children while about 22 % had 4 or more children. A study done by Bhagwan*et al* (Bhagwan, 2007) showed that about 39 % of the respondents had 3 or fewer children while about 61 % had 4 or more children. Ghazala *et al* in a similar study done in Islamabad Pakistan found that 45% of the women had 4 or less children while 55% had 5 or more children (Ghazala, 2013). These studies explain why India and Pakistan are so heavily populated.

The study further revealed that most respondents had less than 2 recent sexual partners which was comparable to a study by Mabeya*et al* in which most respondents had less than 2 recent sexual partners (Mabeya, 2012) and Syrjanen*et al* in which most respondents had less than 2 recent sexual partners (p=0.001) (Syrjanen, 2005). This study however contrasted with another one done by Were *et al* where the majority of therespondents actually had more than 2 recent sexual partners (Were, 2010). The reason for this difference could be the different cultural inclinations of the inhabitants of the two areas whereby in the latter polygamy is widely acceptable

The positivity of Pap smear in this study was 11.5 %. This was far below the estimated national positivity of 43% (MOH, 2012). This big difference may have been caused by the failure of the infected women to turn up in large numbers for screening. However the positivity compared favourably with a study done by Rana *et al*, in which the positivity of Pap smear was found to be 12 % (Rana, 2010). A study done in Webuye found a positivity of 22.7% in a similar population (Laktabai, 2009) while a similar study done by Hend S Saleh at the faculty of Medicine of the University of Zagazig, Egypt showed the positivity of Pap smear to be only 4 % (Saleh, 2014). This very low positivity in Egypt could be explained by the fact that Egypt is an Islamic country and as such the women are loyal to the Islamic laws that forbid any form of sexual activity before marriage.

There was no association between age in years and the occurrence of abnormal Pap smear results in the study population. Fariba*et al* also found no association between age and occurrence of abnormal Pap smear in a study of 2 years follow up of referral patients with abnormal Pap smear (Fariba, 2015).

The number of recent sexual partners and Pap smear results were not correlated in the study population. This contradicted Berrington de Gonzales *et al* study that found that relative risk of invasive cancer was increased with increased number of sexual partners, younger age at first intercourse (Berrington, 2007).

The Government of Kenya recommends use of visual inspection tests as the primary screening tests for cervical cancer while Pap smear may also be used where it is available.

The positivity of VIA in this study was 3.8 %.This contradicted results of a study done by Laktabai in which the percentage of tests with positive results was 28.2 % (Laktabai, 2009). The positivity of VILI in the study was also 3.8%. This also contradicted results of a study done by Sankaranarayanan *et al* in which the percentage of VILI tests with positive results was 17.8 % (Sankaranarayanan, 2003). This apparent low positivity in the visual inspection methods may have been caused by lack of standardized training to Health Care Workers and also due to lack of quality control methods for visual testing

There were more abnormalities detected on Pap smear than on VIA/VILI (11.5% vs. 3.8%). This contrasted findings by Ghazala *et al* that show that visual inspection tests have a higher sensitivity than Pap smear and can replace Pap smear as a primary screening tool for universal screening (Ghazala, 2013). In another study by Ghosh *et al*, 13% of the patients were found to be positive by VIA and 11.71 % were positive on VILI. The Pap smear was abnormal in 3.71 % (Ghosh, 2012).

This apparent contradiction in the test characteristics of VIA and VILI indicate that they are currently not suitable alternate approaches to cervical cytology in Murang'a District Hospital.

CONCLUSION

There was no agreement between Pap smear and VIA/VILI in detecting suspected cervical abnormalities. Pap smear detected more abnormalities than VIA/VILI and therefore the possibility of HIV positive women visiting reproductive health clinic and leaving without cervical abnormalities being detected could not be ruled out.

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