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HPV DNA TESTING AND PAP SMEAR CYTOLOGY CO-TESTING AS A 'TEST OF CURE' IN PATIENTS PREVIOUSLY TREATED FOR CERVICAL LESIONS BY LEEP AT KENYATTA NATIONAL HOSPITAL

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HPV DNA TESTING AND PAP SMEAR CYTOLOGY CO-TESTING AS A 'TEST OF CURE' IN PATIENTS PREVIOUSLY TREATED FOR CERVICAL LESIONS BY LEEP AT KENYATTA NATIONAL HOSPITAL

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ABSTRACT

***Background*: HPV infection is a pre-requisite for the development of the majority (99.7%) of precancerous cervical lesions. Treatment of cervical precancerous lesions reduces the risk of invasive cervical cancer by 90%; however, treated women still have five times risk of invasive cancer compared to women who have always had a normal Pap smear, thus special follow-up measures are critical to reduce these risks.**

***Objective:* To determine the utility of co-testing by conventional Pap smear and HPV testing as a 'test of cure' in patients previously treated for cervical lesions by LEEP at KNH.**

***Design*: Cross sectional descriptive study.**

***Setting:* Kenyatta National Hospital and KAVI molecular laboratory.**

***Subjects*: Women on follow for cervical lesions post LEEP treatment.**

***Results*: Out of the 25 participants, 22(88%) had a report of NILM while 3(12%) had a report of ≥ASCUS). 16 (64%) were positive for HPV. HPV 56 was the commonest HPV subtype detected in 11 patients (41%). The Cohen's Kappa correlation between Pap smear and HPV DNA test not statistically significant=0.143, 95% CI: -0.17 to 0.46, p=0. 166.There was no statistically significant association between HIV status and pap smear findings post LEEP, X^2=0.711, p=0.399**

***Conclusions*: Co-testing with HPV DNA testing and Pap smear is a useful approach to stratify women with no cytological abnormalities according to their risk of residual disease**

INTRODUCTION

Cervical cancer is the second commonest cancer among women worldwide (1). Approximately 80% of cervical cancer related deaths occur in developing

countries (1). This is attributable to the general unavailability of sound and effective screening programs in these settings. Approximately 68% of the estimated 33.3 million individuals living with HIV/ AIDS in the world live in sub- Saharan Africa (2). These areas with high HIV prevalence rates are also burdened with high cervical cancer rates(3).

HIV infected women are at an increased risk of infection with HPV, in addition, studies have shown that HIV infected women have higher prevalence of HPV, higher incidence of HPV, higher HPV viral load, longer persistence of HPV, higher likelihood of multiple HPV subtypes and greater prevalence of oncogenic subtypes compared to HIV negative women(4,5).There is also increased persistence and recurrence after treatment in HIV positive patients with certain studies documenting a recurrence >50% (6).

Treatment of cervical precancerous lesions reduces the risk of invasive cervical cancer by 90%; however, treated women still have five times risk of invasive cancer compared to women who have always had a normal Pap smear (7,8). Thus it is essential to have special follow-up strategies to reduce these risks. Currently follow- up protocols in KNH involves screening with Pap smears only; which have their own limitations. Pap smear has low sensitivity because detectable cytological changes are not always present after infection with HPV.

In addition, the low sensitivity could be due to other reasons such as inadequate sampling, poor quality of smears due to obscuration by inflammation and subjectivity in the interpretation of the Pap smears. The incorporation of high risk HPV genotype testing in addition to Pap smear cytology testing is the approach with the most potential to increase the efficiency and effectiveness of screening in this group of women.

MATERIALS AND METHODS

Study Site: KNH Clinic 66, Kenyatta National Hospital Cytology laboratory and KAVI molecular laboratory. Study period: July- November 2016

Study design: Cross sectional descriptive study.

Ethical considerations: Ethical approval was sought from KNH/UoN ethics committee (Protocol number: P138/02/2016).

Study population: Women on follow for cervical lesions post LEEP treatment. Inclusion criteria

Women previously treated by LEEP

Women who gave consent to participate in the study

EXCLUSION CRITERIA

Women who have had treatment with 6 months of the study. Women with cervical lesions without history of treatment. Patients treated by other methods e.g.cryotherapy or total abdominal hysterectomy Those who decline to give consent.

Sampling methods: Consecutive and snowballing

Specimen processing: Samples for convention Pap smears and HPV were collected using respective brushes and preserved immediately. The alcohol fixed Pap smears were stained using the H&E protocol and reported using the 2014 Bethesda system for reporting cervical smears(9). HPV DNA testing was done using the PCR, REAL TIME. The Pap smears were stained using the Papanicolaou staining procedure in the cytology laboratory. The HPV DNA testing was done in KAVI molecular laboratory by a technologist and the principal investigator.

Statistical analysis: Cohen's kappa test was done to determine the agreement between Pap smear results and HPV results. Chi square test was used to determine associations between HIV status with Pap smear results post LEEP. Chi square test was used to determine associations

between HIV status and HPV positivity post LEEP A p- value below 0.05 was regarded as statistically significant.

RESULTS

Table 1 shows the Pap smear findings. A minority of the women, 3 cases (12%) had abnormal Pap smear cytological findings (ASCUS and above). High grade squamous intraepithelial lesion (HSIL), Low grade squamous intraepithelial lesion (LSIL) and atypical squamous cells cannot exclude High grade squamous intraepithelial lesion (ASC-H) contributed a single case (4%) each.

Table 1

Pap smear findings

Result	n=25	%
NILM	22	88
LSIL	1	4
ASC-H	1	4
HSIL	1	4

Figure 1 shows the HIV status of the study participants. Out of the 25 participants, eleven (44%) were HIV positive and fourteen (56%) were HIV negative.

Figure 1

HIV status of study participants

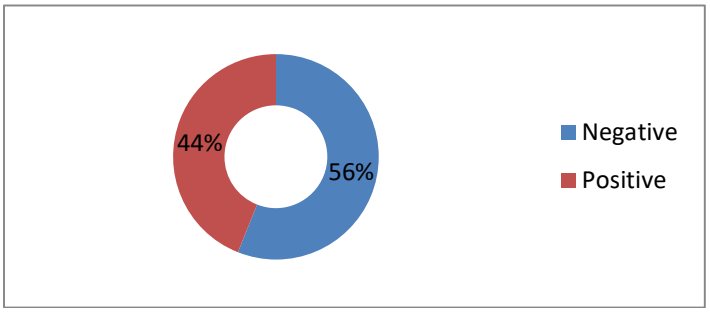

Table 2 shows High risk HPV test results. Most of the women were positive for high risk HPV on follow up (16 cases) (64%) and a few were negative (9 cases) (36%). Single and multiple HPV infections equally distributed each with 8 cases (50%).

Table 2:

High risk HPV test results

HPV genotypes	n=27	%
16	1	3.7
18	2	7.4
31	1	3.7
33	0	0
35	1	3.7
39	1	3.7
45	0	0
51	6	22.2
52	1	3.7
56	11	40.7
58	0	0
59	0	0
68	3	11.1

Single vs. multiple nfection	n=16	%
Single infection	8	50
Multiple infections	8	50

Table 3 shows the cross tabulation of HPV and Pap smear results. The majority of the women thirteen (52%) were positive for HPV but without any a detectable cytological abnormality. All women with abnormal Pap smear findings (three) (12%) were also positive for high risk HPV. Cohen Kappa test was run to determine if there was an agreement between Pap smear and HPV results. There was poor (non to slight) agreement between the tests, k=0.143, 95% CI: -0.17 to 0.46, p=0.166 (p>0.005).

Table 3

Cross tabulation of HPV results and Pap smear

Pap smear results	HR-HPV pos	HR-HPV neg	Total	Cohen's Kappa(k)	p- value
NILM	13	9	**22**	0.143	0.166
Abnormal(≥ ASCUS)	3	0	**3**		
Total	16	9	25		

Table 4 shows the cross tabulation of HIV status and Pap was done to determine if there is an association between HIV smear findings post LEEP. Cervical lesions were more status and Pap smear findings. There was no statistically common in HIV positive patients 2 cases (66.6%) compared significant association HIV status and pap smear findings to HIV negative patients 1 case (33.3%). A Chi- square test post LEEP, X^2=0.711, d.f= 1, p=0.399 (p>0.005).

Table 4

Cross tabulation of HIV status and Pap smear findings post LEEP

Pap smear results	HIV neg	HIV pos	Total	Pearson value	p-value
NILM	13	9	22	0.711	0.399
Abnormal (≥ ASCUS)	1	2	3		
Total	14	11	25		

Table 5 shows the cross tabulation of HPV positivity between HIV positive and HIV negative women was comparable each accounting for 63.6% and 64.3% respectively.

Table 5

Cross tabulation of HIV status and HPV positivity

HPV results	HIV neg	HIV pos	Total	Pearson value	p-value
Negative	5	4	9	0.001	0.97
Positive	9	7	16		
Total	14	11	25		

A Chi- square test was done to determine if there is an association between HIV status and HPV positivity. There was no statistically significant association HIV status and HPV positivity post LEEP, X^2=0.001, d.f= 1, p =0.973 (p>0.005.

Table 6

Table 6 shows the comparison of diagnosis pre and post treatment. The majority of patients with abnormal results (88%) had successful treatments

		Post treatment		
		Neg	Pos	Total
Pre-treatment	normal	1	0	1
	abnormal	21	3	24
	Total	24	3	25

DISCUSSION

Co-testing with HPV DNA testing and Pap smear has proved to be a useful approach to stratify women with no cytological abnormalities according to their risk of residual disease which improves the efficacy of screening programs in this group of women. A Cohen Kappa correlation was done to ascertain the agreement of HPV results and Pap smear results.

However, the test was not statistically significant (k=0.143, p=0.166). This can possibly be because most HPV infections detected were not yet associated with any cytological changes. This makes HPV testing in addition to Pap smear testing a useful and valuable combination as it helps to stratify women according to their risk for future recurrent disease.

In this study, follow up of women post LEEP treatment revealed that 64% of women were HPV positive and 12% had ASCUS or more severe cytological abnormalities. The prevalence of cytological abnormalities was comparable to those reported by Gosvig CF et al in Denmark in which they reported 17% of women with cytological abnormalities post LEEP treatment(10). However HPV positivity in these two studies differs significantly with 64% in our study against 48%. This difference can partly be explained by earlier follow in that study (mean=3.4 months) compared to this study (mean=18 months).

The delay in follow up in this study could have accounted for the higher HPV positivity due to new infections. This study showed that cervical lesions after treatment are more common in HIV positive women (1.8 %) compared to HIV negative women (0.7 %). This is consistent to the one reported by Chirenje M.Z et al in Zimbabwe which showed cervical lesions in 3.8% and 1.8% respectively (11).

These results are also supported by results of a study by Heard et al who reported that 54% of HIV positive women previously treated for cervical lesions had residual or recurrent disease at 36 months (12). However, a Chi- square test revealed no statistically significant association between HIV infection and presence or absence of cervical lesions (p=0.399). HPV positivity after LEEP in HIV positive patients was 63.6 %. This value was comparable to findings by Gingelmaier et al which showed a positivity rate of 57 %(13). HPV positivity in HIV negative patients was 64.3% in our study.

This figure is higher compared to figures reported by Kreimer AR et al and Kocken M et al which ranged from 10-37% (5,6). This may be attributable to the detection of HPV at a fixed period post treatment in those studies unlike ours which had various periods with a mean of 18 months' post treatment. This could explain the higher rates of HPV positivity possibly due to new infections rather than persistent infections.

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REFERENCES

1. World Health Organisation (WHO) international Agencyfor Research on Cancer iarc monographs and the evaluation of carcinogenic risk to humans. IARC. 2007;45–80.
2. UNIAIDS report on the global AIDS pandemic 2010. UNIAIDS. Global report: 2010.
3. Parkin D, Ferlay J H-CM et al. Cancer in Africa: Epidermiology and Prevention. IARC Sci Publ. 2003;153.
4. Clifford GM, Goncalves MA FS et al. For the HPV and HIV Study Group (2006) Human papillomavirus types among women infected with HIV: a meta-analysis. AIDS. 2006;20:2337–44.
5. Vuyst H De, Mugo NR, Franceschi S, Al et al. Residual Disease and HPV Persistence after Cryotherapy for Cervical Intraepithelial Neoplasia Grade 2/3 in HIV-Positive Women in Kenya. PLoS One. 2014;9(10).
6. Tebeu PM, Major AL MP et al. The recurrence of cervical intraepithelial neoplasia in HIV-positive women. Int J STD AIDS. 2006;17(8):507–11.
7. Kreimer AR, Guido RS SD.Human papillomavirus testing following loop electrosurgical excision procedure identifies women at risk for posttreatment cervical intraepithelial neoplasia grade 2 or 3 disease. Cancer Epidemiol Biomarkers Prev. 2006;15:908–15.
8. Kocken M, Helmerhost TJ BJ et al. Risk of recurent high grade cervical intraepithelial neoplasia in Maharashtra. Lancet Oncol. 2011;12:441–50.
9. Solomon D NR. The bethesda system for reporting cervical cytology definations,criteria,and explanatory notes. 2nd ed. Springer; 2008. 89-109
10. .Gosvig CF, Huusom LD DI. Role of human papillomavirus testing and cytology in follow up after conization. Am J Obs Gynecol. 2015;94(4):405–11.
11. Chirenje ZM, Rusakaniko S, Akino V MM. A randomised clinical trial of loop electrosurgical excision procedure (LEEP) versus cryotherapy in the treatment of cervical intraepithelial neoplasia. J Obs Gynaecol. 2001;21(6):617–21.
12. Heard I, Potard V FH. High rate of reccurrence rate of cervical intraepithelial neoplasia after surgery in HIV positive women. J Acquir Immune Defic Syndr. 2005;39:412–8.
13. Gingelmaier A, Grubert T, Kaestner R, Mylonas I, Weissenbacher T BF et al. High recurrence rate of cervical dysplasia and persistence of HPV infection in HIV-1-infected women. Anticancer Res. 2007;27(4A):1795–8.