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Clinical Study

Implications of Post-LLETZ "Treatment Failure" for Further Management of HIV-Infected Women

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Background. Since the preconisation presence of high-risk human papillomavirus (HR-HPV) is the main determinant of the risk of progression of preinvasive lesions; the state of the excision margins could be of less importance. Relatively little is known about the effect of human immunodeficiency virus (HIV) infection on the relation between the states of the excision margins. *Methods*. We compared 120 HIV-infected and 139 HIV-uninfected women who underwent a hysterectomy after large loop excision of the transformation zone (LLETZ) for abnormal Pap smear. *Results*. The excision margins had been reported negative in 21.7% of infected and 7.8% of uninfected cases ($P = 0.03$). Three (11.5%) of 26 negative margins in HIV-infected and 2 (18.2) out of HIV-uninfected cases were falsely negative as evidenced on hysterectomy specimens ($P = 0.73$). The persistence rate of the initial lesion was similar in both groups ($P = 0.20$). The persistence rate with highly active antiretroviral treatment (HAART) was similar to untreated patients ($P = 0.20$). The progression rate from low-grade to high-grade preinvasive lesions was higher in HIV-infected than HIV-uninfected women ($P = 0.027$). *Conclusion*. HIV-infected women with incomplete excision margins after LLETZ are at higher risk of progression of residual preneoplastic lesions.

1. Introduction

The prevention of cervical cancer is twin-pronged: primary (i.e., vaccination and life style) and secondary (i.e., screening and treatment). Many screening strategies are still under investigation and awaiting validation. They comprise cytology and/or human papillomavirus (HPV) testing, visualization of the cervix (naked eye, colposcopy, and cervicography), and excision or destruction of preinvasive lesions [1]. Destruction (by cryotherapy or laser) has the disadvantage of lacking "gold standard" biopsy confirmation but the advantage of on the spot treatment (see and treat) [2]. This is of special importance in low-resource settings (LRS). Excision methods are many: cold knife conisation (CKC) and thermal excision called either large loop excision of the transformation zone (LLETZ) or loop electrosurgical excision procedure (LEEP). Both allow for histopathological diagnosis although, as opposed to CKC, they have the possible disadvantage of burn artifacts of the excision margins. The state of the excision margins (healthy or involved by the preneoplastic process) is, arguably, a prognostic indicator and a guide to further follow-up [3].

LRS like sub-Saharan Africa carry the double burden of inexistent or inefficient screening programs and high cervical cancer and human immunodeficiency virus (HIV) endemicity [1]. Data about screening, prevalence of preneoplasia, and "see-and-treat" strategies are relatively limited. "See-and-treat" strategies are mostly implemented using destructive methods (i.e., cryosurgery) [2]. Therefore, potential information provided by the gold standard of colposcopy-directed biopsy/LLETZ/LEEP/CKC is lacking.

It has been suggested that the two main predictors of persistent cytological abnormalities among HIV-infected women are post-LLETZ excision margin involvement and the level of immunodepression as expressed by the CD4+ T-cell count [4–7]. There is also some evidence that post-LLETZ margin involvement might be more frequent in HIV-infected than HIV-uninfected women [8, 9]. However, local data have not confirmed this [5]. Because of these contradicting data

Table 1: Comparative clinico-pathologic data: HIV-infected versus uninfected.

Variables	HIV-infected $N = 120$	HIV-uninfected $N = 139$	t	P
Age (years)	39.5 ± 6.5 [39.0] (22.0–58.0)*	46.3 ± 10.9 [45.0] (25.0–77.0)	6.0	<0.0001
Weeks between LLETZ and hysterectomy	28.0 (2–96)**	18.0 (3–64)		0.0016
N reported positive margins	94 (78.3)***	128 (92.1)		0.03
CIN1 on hysterectomy or repeat LLETZ	5 (4.2)	8 (5.7)		0.18
CIN2+	106 (88.3)	121 (87.1)		0.28
Microinvasive cancer	9 (7.5)	10 (7.2)		0.06
Regression to ≤CIN1	20 (16.7)	39 (28.1)		0.035
Progression to ≥CIN2+	24 (20.0)	3 (2.1)		0.027
Persistence	76 (63.3)	97 (69.8)		0.10

*Values are mean ± SD [Median]; **Values are median (range); ***Values are numbers (%).

we investigated the status of LLETZ specimen margins in HIV-infected and HIV-uninfected women in the Limpopo province of South Africa.

2. Methods

The study was carried out at the histopathology division of the National Health Laboratory Service, Polokwane, Limpopo province. All consecutive LLETZ cases received in 2013 were collected prospectively. All participants underwent HIV screening. The following information was collected from the histology request form and laboratory database: age, HIV status, CD4+ T-cell count (when available), and pre-LLETZ cytology findings. The LLETZ procedures were indicated for abnormal Pap smears (≥low-grade squamous intraepithelial lesion). Repeat LLETZ and hysterectomies were carried out for reported incomplete excision of preinvasive lesions on the initial LLETZ. In the HIV-uninfected group, 4 (2.9%) had a repeat LLETZ; 6 (5.0%) HIV-infected patients had a repeat LLETZ. All of the others had a post-LLETZ total hysterectomy. For repeat LLETZ cases the pathology of the first excision was compared with repeat procedure.

The lesions were classified as low-grade cervical intraepithelial neoplasia (CIN1), high-grade CIN2+ (including carcinoma in situ), and invasive cancer (IC). Regression was defined as the disappearance of a previous CIN or a downgrade of the initial lesion. Persistence was defined as a residual lesion of same grade as initially found. Progression was defined as worsening of the severity compared to the initial diagnosis.

The specimens were measured; step sections were embedded. The average length and thickness were 2.5 cm ± 0.9 (median 2.0) and 0.9 ± 0.5 (median 1.0), respectively. An average of 8.6 ± 3.5 (median 9.0) sections was cut per LLETZ specimen. Four-micron sections were stained with hematoxylin and eosin.

The state of the excision margins was assessed and compared with the histopathological findings on the ensuing procedure: total hysterectomy or repeat LLETZ for previous incomplete LLETZ. Positive margins were reported regardless of their number (i.e., exocervical, endocervical, and/or deep).

The time elapsed between the first and second procedure was recorded. In case of repeat LLETZ the time was measured between the first and second procedure. All cases were handled anonymously.

Statistical analysis was carried out using column statistics, Student's t-test for continuous variables, and rank sum test for nonparametric variables. The level of statistical significance was set at $P < 0.05$.

3. Results

One hundred twenty patients were HIV-infected and 139 HIV-uninfected. Fifty (41.7%) were on highly active antiretroviral treatment (HAART). The CD4+ T-cell count was known in 50 cases: median 279.0 (range: 6.0–964.0). Seventeen (37.0%) had a count below 200/mm^3 and 7 (15.2%) above 500. The median CD4+ T-cell count of 15 women on HAART was 280.0 (range: 16.0–964.0) and 299.0 (range: 6.0–842.0) in untreated patients ($P = 0.47$).

Table 1 shows that the HIV-infected patients were significantly younger than the HIV-uninfected patients ($P < 0.0001$). The time elapsed between the first and second procedure was longer in the HIV-infected patients than their uninfected counterparts ($P = 0.0016$). The prevalence of histopathological reported positive margins was higher in the HIV-uninfected group than HIV-infected group ($P = 0.03$). There was no difference in distribution of biopsy-diagnosed pathology between the two groups. The regression rate was higher in HIV-uninfected patients ($P = 0.035$). The progression rate was higher in HIV-infected group ($P = 0.027$). The persistence rate was similar in both groups ($P = 0.10$). The persistence rate was 30 (57.7%) out of 52 on HAART and 47 (69.3%) out of 60 untreated ($P = 0.20$).

4. Discussion

The reported rates of positive margins after conisation vary widely from 5.0% or less to up to more than 50.0% with

an average around one-third [5, 10–18]. The variations are attributed to instrumentation and operator variability [10, 16, 19–26]. The advantage of LEEP/LLETZ is that it is a less invasive in-office procedure than CKC that promotes patient's compliance [27]. This is especially relevant in LRS where compliance and loss to follow-up rates are high [5, 27]. The procedure has been shown to be safe in HIV-infected women [7–9, 23].

Traditionally, cervical preinvasive lesions were managed by CKC. The clinician was eager to know the characteristics of the excision margins (clear or involved) to guide further management and follow-up. Knowledge of the role played by low-risk (LR) and high-risk (HR) HPV has brought about a paradigm shift in our understanding and management of cervical preinvasive lesions. For instance, the concept of conisation "with therapeutic intent" is under debate because the natural history of these lesions depends on the clearance or persistence of HR-HPV [3]. In this perspective, it appears that it does not seem to matter that much whether the margins are clear or not [28]. One study, for instance, showed that HPV persistence following LLETZ was associated with smoking and age above 35 years irrespective of margin status [29].

Our rate of positive margins was high but significantly lower in HIV-infected cases. The importance of margin negativity, however, is uncertain and no guarantee of safety [14, 26, 28]. Nonetheless, it appears potentially beneficial to reduce the proportion of diseased margins and the ensuing risk of persistence/progression [18, 30]. The mere fact that conisation eradicates HPV infection in two-thirds of patients indicates that further follow-up is always mandatory [31]. There is growing evidence that preconisation HPV testing strongly predicts the behavior of preinvasive lesions. The risk of progression is low in the absence of HR-HPV and high in their presence especially with positive excision margins [7, 17, 32, 33].

Both local and our data show that the HIV status does not significantly affect the characteristics of the excision margins [5]. The fact remains that regardless of the excision margins follow-up is necessary. This should be done with Pap smear and/or HPV DNA testing [30, 33–36]. Current data show that HR-HPV testing has higher sensitivity than cytology with a similar specificity; therefore, some recommend HR-HPV DNA testing (with Hybrid Capture II, or low-cost/low-tech tests) as the preferred follow-up test [28, 36]. In LRS, low-cost/low-tech tests have been successfully validated [36]. An alternative could be $p16^{INK4a}$ immunohistochemistry, a surrogate biomarker of HR-HPV [37, 38].

Finally, one may wonder whether HIV-infected women require a specific management and follow-up of preinvasive lesions. The evidence is still limited [8]. Lehtovirta et al. found no difference in recurrence rate after positive margins [39]. Nappi et al., on the contrary, found that immunodepression, as evidenced by CD4+ T-cell counts below 200 mm^3, increased the risk of preinvasive lesions [4]. This concurs with South African findings that the presence of disease at both margins and CD4+ T-cell count were the most important predictors [5].

5. Conclusion

The progression rate from low-grade to high-grade preinvasive lesions was higher in HIV-infected than HIV-uninfected women. HIV-infected women with incomplete excision margins after LLETZ are at higher risk of progression of residual preneoplastic lesions especially if the CD4+ T-cell count is below 200/mm^3.

Conflict of Interests

The author declares no conflict of interests.

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