ORIGINAL RESEARCH ARTICLE

Genital Tract Abnormalities among Female Sex Workers Who Douche with Lemon/Lime Juice in Nigeria

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ABSTRACT

Vaginal douche products have been associated with cervical cancer. We examined female sex workers (FSWs) in Nigeria who douche with lemon or lime juice and compared the findings with that of nonusers. We obtained Pap smears and performed colposcopy of the vulva, vagina and cervix. A total of 374 FSWs comprising 81 Lemon users (LUs) and 293 non lemon users (NLUs) were examined. Their mean age was 27.8 ± 6.7 (range 16-63) years. At colposcopy, 17(4.5%) had genital warts [LUs 5(6.2%); NLUs 12(4.1%); p=0.43], 61(16.3%) had suspected squamous intraepithelial lesions (SILs) [LUs 17(21.0%); NLUs 44(15.0%); p=0.20] and 65(17.4%) had other findings. Pap smear cytology showed that 87(24.6%) had SILs [LUs 26(33.3%); NLUs 61(22.1%); p=0.03]. Lemon/lime use was associated with cervical dysplasia after controlling for HIV status (Adjusted OR=1.8; 95% CI, 1.0-3.0). Our data suggests an association between the practice of douching with citrus juice and cervical dysplasia *(Afr J Reprod Health 2009; 13[1]:37-45).*

RÉSUMÉ

Les anomalies des voies génitales chez les prostituées qui se douchent en se servant du citron ou du jus de limon au Nigéria. Les produits de la douche vaginale ont été liés au cancer du col. Nous avons étudié les prostituées au Nigéria qui se douchent en se servant du jus de limon et nous les avons comparées à celles qui ne s'en servent pas. Nous avons collecté le papanicolaou et nous avons fait la colposcopie de la vulve, du vagin et du col. Au total, nous avons étudié 374 prostituées qui comprennent 81 qui se servent du citron et 293 qui ne se servent pas du citron. Leur âge moyen s'est élevé à 27,8 ± 6, 7 (entre 16 et 63 ans). La colposcopie a montré que 17(4,5%) avaient des verrues génitales [celles qui se servent du citron = 5(6,2%) ; celles qui ne s'en servent pas = 12(4,1%) ; p=0,43], 61(16,3% avaient des lésions intraepitheliales squameuses suspectes (LIS) [celles qui se servent du citron = 17(21,0%) ; celles qui ne s'en servent pas = 44(15,0%) ; p = 0,20] et 65 (17,4%) avaient d'autres résultats. La cytologie du papanicolaou a montré que 87(24,6%) avaient LIS [Celles qui servent du citron = 26(33,3%) ; celles qui ne s'en servent pas = 61(22,19%) ; p = 0,03]. L'utilisation du citron/du jus de limon a été liée à la dysplasie cervicale après avoir contrôlé pour l'état du VIH (OR yiste = 1,8 ; 95% CI, 1,0 – 3,0). Nos données indiquent un lien entre la pratique de se doucher avec le jus de limon et la dysplasie cervicale *(Afr J Reprod Health 2009; 13[1]:37-45).*

KEYWORDS: CIN, Cytology, Douching, Lime, Nigeria

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Introduction

Vaginal douching before or after sexual intercourse is a widespread practice among women of diverse cultures worldwide, and commercial antiseptics, soap with water and salty water are commonly used agents.[1-3] Substances like Nonoxynol-9 (N-9), Krest bitter lemon (soft drink) and lime juice have been demonstrated to have antimicrobial and spermicidal properties and have been considered as candidates for the prevention of pregnancy and sexually transmitted infections.[4-6] Vaginal douching with lemon or lime juice is a common practice among female sex workers (FSWs) in northern Nigeria.[7] Recent pre-clinical research compared the cytotoxicity of lemon and lime juice to that of the spermicide (N-9). When tested on cervical explant tissue, lemon and lime juices caused damage to cells comparable to that of N-9.[8] In sexually abstinent volunteers, douching with lime juice caused dose-dependent epithelial damage to the cervix and vagina.[9] Primate studies have, however, shown that daily intravaginal administration of undiluted lime juice (pH 2.5) for one month does not appear to damage the cervical or vaginal epithelium.[10] Frequent vaginal douching can modify vaginal flora and increase the risk of cervical infections.[11] Our earlier publication showed no association between the prevalence of HIV and other STIs and sex-related vaginal douching with citrus juice.[12] Lysol and other tar-based vaginal douche products were voluntarily removed from the US market over 30 years ago because several studies showed significant association with cervical cancer.[13] The impact of sex-related vaginal douching with lemon or lime juice on cervical dysplasia, which is sexually acquired, has not been determined. We sought to determine if there was any association between a history of vaginal douching with lemon/lime and cervical dysplasia among FSWs in northern Nigeria.

Methods

Advocacy and mobilization

Jos is an urban centre in north-central Nigeria with a population of about one million people. We held separate meetings with brothel managers and representatives of FSWs in the Jos metropolis. The purpose of the study was explained at these meetings in order to seek their cooperation. The FSWs expressed concerns about confidentiality and the financial repercussions of time commitment in public health facilities and so a private health centre with adequate facilities was chosen as the study site in preference to a public health centre.

Study Design and Clinical Methods

Douching with citrus juice is a sex-related practice, therefore, we recruited consenting brothel-based FSWs as subjects for this study. We conducted a cross-sectional observational study of consecutive volunteers. The health talks, pre- and post-test counseling, gynae-

cological examination, colposcopy, genital sample collection and laboratory testing were all done at Solat Women Hospital, Jos, Nigeria.

A written informed consent was obtained from each subject and a structured questionnaire was administered by a trained nurse-counselor. Personal bio-data, information about lemon/lime usage and duration of sex work were obtained. A gynaecologist who was unaware of the subject's responses obtained a Pap smear and conducted colposcopic examination of the cervix, vagina and vulva, before and after application of 3% acetic acid. A Pap smear was obtained as previously described[14] and colposcopy examination was conducted using a Carl Zeiss colposcope NAG2, West Germany. Pap smear slides were examined by a trained cytologist and reported using the Bethesda terminology.[15] At colposcopy, the findings were graded as warts (warty lesion on cervix, vagina and/or vulva), cervicitis and suspected squamous intraepithelial lesions (SILs). Biopsy specimens were taken of suspected SILs. Women with detectable abnormalities were offered appropriate treatment and follow up free of cost. Ethical approval for this study was granted by the Ethical Review Board of the Jos University Teaching Hospital.

Statistical Analysis

Data entry and analysis were done using Epi Info version 3.3.2 (CDC, Atlanta Georgia, USA). Associations between cervical dysplasia and use of lemon/lime variables were tested using odds ratios (OR) with 95% confidence intervals (CI) and the Chi square (X^2) test. Multivariate logistic regression was used to assess the independent effect of lemon/lime use on the risk of cervical dysplasia adjusted for HIV status. A P-value less than 0.05 was considered significant.

Results

Of the general study population of 398 subjects who consented to participate in this study and completed questionnaires, 24 did not return for a Pap smear and colposcopy (they were menstruating at the time of the initial evaluation). Only 1.5% (6/398) of these women had previously had a Pap smear. A total of 374 FSWs comprising 81 Lemon users (LUs) and 293 non-lemon users (NLUs) were recruited and examined. The mean age was 27.8 ± 6.7 (range 16-63) years and 94% of the subjects were Christians and 5.5% Moslems. About half (49%) of the subjects were single while 47% were either divorced or widowed and 3.5% were married. Only 7% were illiterate. Age (P=0.56) and education (P=0.76) were unrelated to lemon/lime use. The duration of sex work (P=0.004) correlated positively with lime use. The characteristics of all subjects who participated in this study are outlined in Table 1.

Lime use (90.4%) was more prevalent than lemon use (14.2%) and the principal reason for douching was to prevent infection (75% of subjects). Among LUs, 12% applied lemon/lime juice only before sex while 54% applied

Table 1: Characteristics of the Study Subjects
Blank, missing data due to refusal of female sex workers to answer question

Variable	Lemon/lime Users (%) n=86	Non-Lemon/Lime Users (%) n=312	P-value
Mean Age ± SD (Range) Years	28.0±7.7 (18-54)	27.4±6.8 (16-63)	0.55
Age Groups			0.56
>15-20	9(10.5)	35(11.2)	
>20-25	35(40.7)	111(35.6)	
>25-30	17(19.8)	94(30.1)	
>30-35	11(12.8)	36(11.5)	
>35-40	9(10.5)	24(7.7)	
>40	5(5.9)	12(3.8)	
Religion			1.0
Christian	80 (95.2)	290 (94.2)	
Muslim	4 (4.8)	18 (5.8)	
Blank	2(2.3)	4 (1.3)	
Education			0.19
Illiterate	7(8.1%)	20(6.5)	
Primary	31(36.0)	112(36.2)	
Secondary	37(43.0)	155(50.1)	
Tertiary	7(8.1)	20(6.5)	
Other	0(0)	2(0.6)	
Blank	0(0.0)	3(0.9)	
Marital status			0.21
Single	31(39.2)	135(51.9)	
Married	2(2.5)	10(3.8)	
Divorced	27(34.2)	70(26.9)	
Separated	0(0)	2(0.8)	
Widowed	19(24.1)	43(16.5)	
Blank	7(8.0)	52(16.7)	
Duration of Sex work*			0.002
<1year	15(17.9)	85(28.2)	
1-5years	49(58.3)	184(61.1)	
>5-10years	17(20.2)	26(8.6)	
>10years	3(3.6)	6(2.0)	
Blank	2(2.3)	11(3.5)	

* Duration of sex work was positively related to lemon or lime juice use ($\chi 2$ for trend P=0.002).

Table 2: The mode, preference and rationale for use of lemon/lime juice for douching by FSW in northern Nigeria (*Blank, missing data due to refusal of female sex workers to answer question)*

Variable	N (%)	95% Confidence Interval
The citrus juice used		
Lemon	8 (9.5)	4.2-17.9
Lime	72 (85.7)	76.4-92.4
Both	4 (4.7)	1.3-11.7
Blank	2 (2.3)	0.7-3.9
Reason for using lemon/lime juice		
To prevent infection	40 (54.1)	42.1-65.7
To prevent pregnancy	4 (5.4)	1.5-13.3
To prevent both infection & pregnancy	15 (20.8)	11.8-31.2
To increase sexual pleasure for your customers	9 (12.2)	5.7-21.8
To increase sexual pleasure for you and your customer	6 (8.1)	3.0-16.8
Blank	13 (15.1)	7.5-22.5
How lemon/lime juice was used for sex		
Apply to outside of the vagina	25 (32.5)	22-44.1
Apply to inside the vagina	43 (55.8)	44.1-67.2
Other	9 (11.7)	5.5-21.0
Blank	10 (11.6)	4.9-18.3
Concentration of lemon/lime juice used		
Neat (undiluted)	21 (25)	16.2-35.6
Diluted in water	59 (70.2)	49.3-79.7
Diluted in other Fluids	2 (2.4)	0.3-8.3
Others	2 (2.4)	0.3-8.3
Blank	3 (3.5)	0.0-7.4
How lemon/lime juice was used		
Before Sex	10 (12.2)	6.0-21.3
After Sex	44(53.7)	42.3-64.7
Both	27 (32.9)	22.9-44.2
Other	1 (1.2)	0.0-6.6
Blank	5 (5.8)	0.9-10.7
Use of lemon/lime juice with customers		
With every customer	37 (45.7)	34.6-57.1
With most customers	5 (6.2)	2.0-13.8
With a few customers	8 (9.9)	4.4-18.5
Have used it in the past	17 (21.0)	12.7-31.5
Other	14 (17.3)	9.8-27.3
Blank		

Table 3: Colposcopy and cervical cytology findings among FSWs in northern Nigeria

Variable/Findings	Lemon/Lime Users	Non-Lemon/Lime Users	Odds Ratio (95% Confidence Interval)	P value
Colposcopy	**n=81 (%)**	**n=293 (%)**		
Normal	50 (61.7)	190 (64.8)		
Cervicitis	13(16.0)	52 (17.7)	0.89 (0.43-1.80)	0.721
*Warts	5(6.1)	12 (4.1)	1.54 (0.46-4.90)	0.427
Suspected SILs	17 (20.9)	44 (15.0)	1.50 (0.77-2.92)	0.198
Cytology	**n=78 (%)**	**n=276 (%)**		
Normal	48 (61.5)	193 (69.9)		
ASCUS	4 (5.2)	22 (8.0)	0.73 (0.18-7.30)	0.579
LSIL	10 (12.8)	30 (10.9)	1.38 (0.56-3.12)	0.417
HSIL	16 (20.5)	31 (11.2)	2.13 (1.03-4.40)	0.025
LSIL/HSIL	26 (33.3)	61 (22.1)	**1.76 (1.0-3.10)	0.042

* Warts frequently involved the cervix, vagina and vulva.
**Adjusted for HIV status

it only after sex. About half (49%) of LUs reported discomfort or pain with use. Colposcopy showed 17(4.5%) subjects with genital warts [LUs 5(6.2%); NLUs 12(4.1%); p=0.43], 61(16.3%) with suspected SILs [LUs 17(21.0%); NLUs 44(15.0%); p=0.20] and 65(17.4%) had findings suggestive of chronic cervicitis (Table 3). We did not find vulval or vaginal abrasions at colposcopy. Cytology of Pap smears showed that 87(24.6%) had SILs [LUs 26(33.3%); NLUs 61(22.1%); p=0.03]. Lemon/lime use was associated with cervical dysplasia after controlling for HIV status (Adjusted odds ratio=1.76; 95% CI, 1.0-3.0). In our recent report [12], HIV prevalence for LUs and NLUs were 48.8% and 48.2% respectively (OR 1.0; 95% CI 0.6-1.2, p=0.9427). Abnormal cytology was not associated with increasing duration of lemon/lime use (P= 0.20 for trend).

Discussion

The main reason why FSWs in this study used citrus juice for sex was to prevent infection. The concentration of citrus juice used, method of application and frequency of use could not be standardized because of obvious limitations in the study design warranted by ethical barriers. However, the following findings deserve careful consideration.

Cytology of Pap smears showed that lemon/lime use was associated with

cervical dysplasia (SILs) after controlling for HIV status. On the one hand, the prevalence of SILs among FSWs in this study (87/354; 24.6%) was higher than previously reported for women in the same city (7.7%).[16] On the other hand; there is a suggestion that the practice of douching with citrus juice may be a risk factor for cervical dysplasia. To the best of our knowledge, this association has never previously been reported and it deserves further evaluation in communities where this practice is common. It is also noteworthy that although Pap smear services were available in the local tertiary health facility, only 1.5% of the FSWs had previously had a Pap test. The absence of a population-based cervical screening program in Nigeria is putting the lives of many women at risk. In 1997, Zhang and colleagues [17] reported the results of a meta-analysis of 6 studies of vaginal douching and cervical cancer. Among women who douched at least once per week, the pooled adjusted relative risk was 1.86 (95% CI = 1.29-2.68). Of note, this statistically significant difference was for vaginal douching with any product. Cervical human papilloma virus (HPV) infection is widely accepted as a precursor to dysplasia but in this study, we found no difference in the prevalence of genital warts between LUs and NLUs. The aetiology of cervical cancer is multifactorial. Lysol and other tar-based vaginal douche products were voluntarily removed from the USA market over 30 years ago because several studies showed significant association with cervical cancer.[13]

It has been demonstrated that even a 20% concentration of either lemon or lime juice had the effect of inhibiting Lactobacilli [8] and that frequent vaginal douching interferes with the normal vaginal flora thereby predisposing to cervical infections and pelvic inflammatory disease (PID).[2,11,18] This study showed no difference in prevalence of colposcopically detected evidence of chronic cervicitis between lemon/lime users and non users. In our earlier report on this cohort of FSWs, we showed that sex-related douching with lemon or lime juice was not a risk factor for HIV and other sexually transmissible infections. [12] There was however an increased prevalence of bacterial vaginosis although this did not reach statistical significance. [12]

When tested on cervical explant tissue (cells obtained from routine hysterectomies that have been kept alive in lab cultures), results indicated that the lemon and lime juices caused damage to cells comparable to that of N-9. [8] Douching with lime juice caused dose-dependent epithelial damage to the cervix and vagina in sexually abstinent women volunteers [9] but among FSWs who used lime douches for sex, our study found no colposcopically detectable evidence of abrasions or bruises. Whether alkaline semen plays a role in modulating the impact of lemon/lime juice on the epithelium of the genital tract remains a matter of conjecture.

Our study had several limitations. We relied on self reporting by subjects and

have no means of verifying claims or controlling for other factors like degree of dilution of lime juice, timing of douching and condom use. We were unable to control for frequency of condom use, timing of douching and degree of citrus juice dilution. Because of the cross-sectional nature of the study, we cannot be confident that use of lime juice preceded the development of cervical dysplasia. Confounding by association may have arisen if use of lime juice is associated with other practices that increase the risk of cervical dysplasia.

There is a widespread habit of vaginal douching among FSWs in Africa and citrus juice is among the frequently used agents. [2,3,7] Our findings however suggest that sex related douching with citrus juice does not appear to damage genital tract epithelium nor is it associated with increased risk of cervical infections or genital warts. However, further studies to explore the association between douching with lime juice and cervical dysplasia are warranted in communities where this practice is common.

Acknowledgements

We are grateful to the women who participated in this study; and the research staff involved in the field work, counselling and testing of biological samples. We are thankful to the Medical Director and Staff of Solat Women Hospital, Jos for their cooperation and contribution. The strong support and encouragement received from Brian Haill are appreciated. This study was supported by public donation to the Mary Magdalene project, www.aids.net.au.

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