Female genital mutilation and non-invasive cervical abnormalities and invasive cancer in Senegal, West Africa: A retrospective study

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Abstract

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We sought to investigate the relationship between exposure to female genital mutilation (FGM) and the main types of invasive cervical cancer (ICC), notably squamous cell carcinoma and adenocarcinoma.

Background: Cervical cancer is the fourth most common cancer in women worldwide. In sub-Saharan Africa, it accounts for 22% of all cancers reported in women. Although preventable, the absence of regular screening and appropriate treatment for cervical cancer leads to late diagnosis and high mortality in African women. Female genital mutilation or cutting (FGM/C), also known as female circumcision, is a traditional practice that has been documented in 29 different countries, mainly in Northern and West Africa, areas of the Middle East, and some countries in Asia. Its long-term consequences include recurrent infection, urinary incontinence, pain with intercourse, complications during childbirth, and psychological effects. In Senegal, according to the 2014 Demographic Health Survey (DHS), an estimated 25% of women and girls, aged 15-49, have experienced FGM.

Methods: We performed a secondary analysis using combined data from six research studies conducted in and around Dakar, Senegal from 1994 to present. Our study subjects included women who presented to outpatient clinics who were mostly asymptomatic but were screened for cervical cancer, and women with cancer symptoms who were referred to the Hopital Le Dantec for cervical cancer treatment. We

conducted separate logistical regressions to estimate the odds ratio (OR) and 95% confidence intervals of having cervical abnormalities and of having ICC in women with FGM, each compared to a reference group of women with no cervical abnormalities.

Results: After adjusting for age, children, HIV, CSW, smoking, marital status, ethnicity, visit year, education, sex partners, and age at first pregnancy, women with ICC were 2.24 times more likely to have had FGM (95% CI, 1.12-4.49). This association was strongest in our analysis restricted to HPV-positive women. We found that HPV-positive women with ICC were 3.20 times more likely to have FGM (95% CI 1.29-7.96). We did not find a significant association between women with cervical abnormalities and FGM (OR=1.11; 95% CI, 0.82-1.49). Similarly, there was no association between HPV and FGM (OR=0.82; 95% CI, 0.55-1.24) in analyses restricted to women who were negative for cervical abnormalities and ICC.

Discussion: In our sample of Senegalese women with and without cervical abnormalities and invasive cancer, FGM was strongly associated with ICC. Our results suggest that while FGM may not be a significant risk factor for HPV or cervical abnormalities, it could increase risk for ICC, especially in the presence of HPV infection.

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LIST OF ACRONYMS

FGM	Female genital mutilation
OR	Odds ratio
ICC	Invasive cervical cancer
HIV	Human immunodeficiency virus
DHS	Demographic Health Survey
CSW	Commercial sex worker
HPV	Human papillomavirus
STI	Sexually transmitted infection
IARC	International Agency for Research on Cancer
ASCUS	Atypical squamous cells of uncertain significance
LSIL	Low grade squamous intraepithelial lesion
HSIL	Low grade squamous intraepithelial lesion
CIN	Cervical intraepithelial neoplasia
AIS	Adenocarcinoma in situ
CIS	Carcinoma in situ
DNA	Deoxyribonucleic acid

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INTRODUCTION

Sub-Saharan Africa has the highest incidence of cervical cancer in the world, with an estimated 34 out of 100,000 women diagnosed each year according to the World Health Organization [1]. In African women, it is the most common cancer, accounting for 22% of all cancers [1]. Although preventable, the absence of regular screening and appropriate treatment for cervical cancer leads to late diagnosis and high mortality in African women [1]. Worldwide, human papillomavirus (HPV) is the most common sexually transmitted infection (STI), affecting one in five women in the African region [2]. While HPV is recognized as the primary cause of cervical cancer, introduction of the HPV vaccine in low and middle-income countries has lagged behind [2]. Most women infected with an oncogenic HPV type will not develop cancer; the progression to cancer often requires other cofactors [3]. There is evidence to indicate an association between smoking, multiparity, early marriage, and oral contraceptive use and cervical cancer [3]. A study conducted in Mali by Bayo et al. found that women in polygamous marriages had a two-fold increased risk of cervical cancer, and increasing risk associated with an increasing number of wives [4]. Munoz et al. studied pooled data from eight case-control studies of HPV-positive women across four continents and reported a strong association between parity and cervical cancer [5]. The odds ratio for women with seven or more full-term pregnancies was 3.8 (95% CI 2.7-5.5) compared to nulliparous women and 2.3 (95% CI 1.6-3.2) compared to women with one or two full-term pregnancies [5]. Studies have also found that HIV-positive women tend to have more persistent HPV infections, and are not only at a higher risk for cervical cancer, but also develop cancer at an earlier age than HIV-negative women [6, 7, 8].

Female genital mutilation or cutting (FGM/C), also known as female circumcision, is a traditional practice that has been documented in 29 different countries, mainly in Northern and West Africa, areas of the Middle East, and some countries in Asia [9]. While an overall decline has been observed in the last three decades, an estimated 100-140 million women and girls worldwide are thought to be affected [9].

Serious sexual and reproductive health problems are experienced by girls and women who have undergone FGM. Long-term consequences include recurrent infection, urinary incontinence, pain with intercourse, complications during childbirth, and psychological effects [10]. Although the evidence has not shown women who have undergone FGM to be at a greater risk of HIV, it is plausible that the use of unsterilized instruments on girls undergoing FGM together could increase the risk of HIV transmission [11]. In Senegal, according to the 2014 Demographic Health Survey (DHS), an estimated 25% of women and girls, aged 15-49, have experienced FGM, with minimal variation between urban (23%) and rural areas (28%) [12]. FGM is generally performed when girls are young. In Senegal, almost three quarters of women with FGM were cut before the age of 5 years old [13].

The World Health Organization (WHO) has classified the four main types of FGM/C based on invasiveness of the procedure [14]:

- **Type I:** Partial or total removal of the clitoris and/or prepuce. Also known as 'clitoridectomy'.
- **Type II:** Partial or total removal of the clitoris and labia minora, with or without excision of the labia majora. Also known as 'excision' in English. However, in French the term 'excision' refers to all forms of FGM/C.
- **Type III:** Narrowing of the vaginal opening by cutting and bringing together the labia minora and/or the labia majora through stitching. Also known as 'infibulation' and results in near covering of the urethra and vaginal opening. Women must undergo 'defibulation', a procedure which reopens the vaginal orifice for sexual intercourse and childbirth.
- **Type IV:** All other procedures that harm the female genitalia for non-medical purposes, e.g., pricking, piercing, incising, scraping and cauterization. Also known as 'symbolic circumcision'.

Most Senegalese women have undergone Type II excision according to self-reported data from the 2014 DHS [12]. Close to 12% of women who reported that they had been circumcised said they were sewn closed (Type III) and about 23% reported not knowing what type of circumcision had been done [12].

Senegal passed anti-FGM legislation in 1999 with punishment resulting in up to 5 years imprisonment for performing FGM [13]. In its Action Plan 2000-2005, Senegal's Ministry of Family Affairs called for FGM's eradication by 2015. The absence of reliable household level data prior to 2005, however, makes it difficult to assess the country's progress in reducing FGM. At a national level, the proportion of Senegalese women with FGM decreased from 28% in 2005 to 25% in 2014 [12]. In the absence of long-term time trends, the lower prevalence in adolescents (13% among girls aged 14 years old) provides some indication that rates are declining [12].

Clinical and epidemiological studies have established a close association between inflammation and carcinogenesis [15]. Inflammation is the body's natural response to infection and tissue damage and aids in eliminating pathogens and initiating tissue repair. However, chronic inflammation is harmful and predisposes cells for oncogenic transformation thereby increasing the risk of developing cancer [16]. There is evidence that chronic inflammation is an important risk factor for epithelial cancers, in particular [17]. Sexually transmitted infections (STIs), such as Herpes simplex type 2, Chlamydia trachomatis, and Neisseria gonorrhea also cause an inflammatory response and have been shown to increase cervical cancer risk after accounting for infection with high-risk types of HPV [18, 19, 20]. Hawes and Kiviat posit that the chronic inflammatory response that accompanies these infections is likely a common explanatory factor in cervical cancer pathogenesis [17]. It is therefore plausible that women who have undergone FGM could be at greater risk for developing cervical cancer given the life-long complications which include recurrent infections, chronic inflammation, and scarring. The literature on this potential association is nearly nonexistent and establishing a relationship with ecological data is difficult due to the absence of reliable country-level data (Figure 1). According to the International Agency for Research on

Cancer (IARC), eastern Africa is one of the highest-risk regions in Africa for cervical cancer, with an age-standardized incidence mean of 42.7 cases per 100,000 females [21]. FGM also happens to be most prevalent in northeastern Africa, with rates as high as 91% in Egypt and 88% in Eritrea [22]. A country-level comparison of ICC incidence in the countries where FGM is practiced is shown in Figure 1.

The only study of which we are aware that has assessed an association between FGM and cervical cancer was conducted in Mali in 2002 and found a 30% greater odds of developing cervical cancer in women who had undergone FGM [4]. These findings are limited, however, by the study's small sample size (82 cases and 97 controls) and fact that nearly all study subjects had undergone FGM (95% cases and 93% controls). Additional research is needed to better understand the relationship between FGM and cervical cancer. This study aims to overcome the limitations of the previously conducted study in Mali by analyzing a larger sample size and more equally distributed exposure prevalence among study groups.

There are three potential mechanisms that could explain an association between FGM and cervical cancer. They are:

1) Chronic infection, irritation, and inflammation, as a result of FGM, could lead to squamous metaplasia in which non-cancerous cells change to a squamous morphology.
2) Trauma and laceration of genital tissue could increase women's susceptibility to HPV infection, which is a known cause of cervical cancer.
3) Compromised immune system, suffered by women who have undergone FGM, could make it more difficult to clear HPV infections.

From a public health perspective, identification of FGM as a risk factor for cervical cancer could lead to better care and cancer-prevention, in addition to better awareness of FGM's detrimental effects on the health and well-being of girls and women in countries where the practice is common.

This study seeks to assess the relationship between exposure to female genital mutilation (FGM) and the main types of invasive cervical cancer (ICC), notably squamous cell carcinoma and adenocarcinoma. Specifically, we will compare the frequency of FGM in women with ICC (1) and in women with non-invasive cervical abnormalities (2) to the frequency of FGM in women with neither cancer nor cervical abnormalities to determine if women who have undergone FGM are more likely to have ICC and/or non-invasive cervical abnormalities. Our secondary aims are to evaluate the relationship between FGM and cervical cancer in HIV-positive and HIV-negative women and to assess the potential impact of commercial sex work (CSW) on the relationship between FGM and cervical abnormalities.

METHODS

Data collection and study population

We conducted secondary data analysis using combined data from six research studies regarding HIV-1 and/or HIV-2, HPV, and cervical cancer conducted in and around Dakar, Senegal from 1994 to present. A complete description of the study subject recruitment methods and study designs have been previously published [23-28]. Briefly, study subjects include women who presented to outpatient clinics (primary care, family planning, infectious disease, oncology, STD clinics) located in and around Dakar, Senegal, as summarized in Table 1. The aims of the cervical cancer related studies were to investigate the epidemiology of HPV, pre-invasive lesions, and invasive cancer associated with HIV-infection, DNA methylation, and cancer control approaches, while other studies had aims exclusively related to HIV-1 and/or HIV-2 infection; all are described in Table 2. Our criteria for including subjects in the current study were presence of a study subject research record with cytology and/or histology data and a physical exam with indication of whether a woman had undergone FGM.

Women presenting to research clinics in studies 1 – 5 were mostly asymptomatic for cervical cancer symptoms but were screened for cervical cancer, HIV, and HPV infection. Women in study 6 had cancer symptoms and were referred from non-research clinics to the Hopital Le Dantec for cervical cancer treatment. Cervical cancer screening was based on cervical cytology using conventional Pap smears from October 1, 1994, through March 31, 1998. Pap smears were interpreted and classified according to the Bethesda System as unsatisfactory, negative, atypical squamous cells of uncertain significance (ASCUS), low grade squamous intraepithelial lesion (LSIL), high grade squamous intraepithelial lesion (HSIL), carcinoma *in situ* (CIS), or invasive cervical cancer. Beginning April 1, 1998, the monolayer cell preparation system ThinPrep® (Hologic Inc, Marlborough MA) was used to obtain all Pap smears, and diagnoses from these slides were made by the cytopathologist in Seattle. Depending on the study protocol, cervical biopsies were conducted using colposcopy in a subset of study subjects (i.e. in those with high risk HPV or those with cytologic findings of HSIL or worse) to confirm disease presence histologically. In studies 5 and 6, cervical biopsies were taken on all study participants. Representative hemotoxylin-eosin–stained slides were prepared from paraffin-embedded biopsy specimens and were reviewed by the study pathologist. The pathologist had no knowledge of other clinical or laboratory data. Biopsy findings were interpreted by the pathologist as negative, reactive atypical changes, cervical intraepithelial neoplasia (CIN) grades I, II, or III, CIS, or ICC according to World Health Organization criteria.

For this data analysis, we combined study subject histology and cytology results, as shown in Table 3, and reassigned study subjects to three disease categories: negative for cervical abnormalities, non-invasive cervical abnormalities, and ICC. Using histology results as our gold standard, we defined study subjects as having non-invasive cervical abnormalities, hereafter referred to as cervical abnormalities, if histology results indicated CIN1 or CIN2-3 (N=110), carcinoma in situ (CIS) or adenocarcinoma in situ (AIS) (N=21), or atypical squamous cells (ASCUS) or atypical results (N=28). We defined study subjects as having ICC if histology results indicated squamous cell carcinoma (N=304) or adenocarcinoma

(N=20). Study subjects with negative histology were defined as negative for cervical abnormalities (N=233). Among 2,410 study subjects, biopsy samples were not collected (N=1,678) or were unsatisfactory for diagnosis (N=6) for 1,684 study subjects. In these subjects we used cytology results to assign subjects to disease categories. These subjects were defined as having cervical abnormalities if cytology results indicated LSIL or HSIL (N=235), CIS or AIS (N=23), or ASCUS or atypical results (N=182). We defined these study subjects as having ICC if cytology results indicated squamous cell carcinoma (N=22) or adenocarcinoma (N=1). In addition, these study subjects were defined as negative for cervical abnormalities if cytology was negative (N=1,219). In these study subjects that had missing cytology (N=95), we used histology and cytology results from a follow-up visit. Follow-up visits occurred at 4 month intervals for subjects enrolled in longitudinal studies while subjects from other studies with unsatisfactory histology or cytology results had another sample taken immediately. Study subjects with histology results that indicated another pathology (N=10) or those missing follow-up histology and cytology were excluded (N=2).

HPV detection methods and genotyping techniques varied across studies and included DNA testing by polymerase chain reaction (PCR) using reverse line blot (RLB) assays, generic and type-specific probes, and liquid bead microarray assay (LBM) based on Luminex technology, described elsewhere [23, 28]. Depending on the study, cervical cellular samples were tested for up to 38 HPV types.

FGM data in study subject research records indicated yes/no based on pelvic examination by a study physician or nurse midwife (sage femme) and did not include further classification by type. The demographic and socio-economic characteristics of study subjects are summarized in Table 4.

Data analysis

We described the demographic and behavioral characteristics of women with ICC, cervical abnormalities, and negative for cervical abnormalities in our sample. We performed univariable and multivariable analyses to investigate the association between FGM and disease status. We conducted separate logistical regressions to estimate the odds ratio (OR) and 95% confidence intervals, first of having cervical abnormalities, and second of having ICC, in women with FGM compared to a reference group of women with no cervical abnormalities. In our multivariable model we included risk factors for cervical cancer defined *a priori* based on our literature review (2-3, 14-16). These risk factors included: smoking, HIV, having a full-term pregnancy before age 17 years, having multiple full-term pregnancies, and polygamous marriage. Then, we identified additional variables as potential confounders by analyzing the effect of each variable on the univariable relationship between FGM and cervical cancer. Variables that changed the OR by 10% or more were considered confounders and were added to our multivariable model. These variables included: age, ethnicity, number of sex partners, commercial sex worker (CSW) by profession, education, and visit year. We found that religion changed the OR by more than 10%, but 36% of the data was missing (N=858) which precluded adjusting by this variable. We stratified the analyses by CSW and HIV status in order to examine how the relationship between FGM, ICC, and cervical abnormalities is modified by these subject characteristics. We also conducted sensitivity analyses to examine whether results differed in subsets of the study population. Specifically, we removed study subjects referred to Hopital Le Dantec with presumed cancer, thereby restricting the analysis to study subjects selected through screening. Second, we redefined study subjects with ASCUS or atypical results as being negative for cervical abnormalities. Finally, we restricted our analysis to HPV positive study subjects. In additional analyses, we restricted the study population to women negative for cervical abnormalities and studied whether FGM is associated with risk of having HPV. We also divided study subjects with cervical abnormalities into low grade (including LSIL/CIN1, ASCUS, or atypical) and high grade (including HSIL/CIN2-3, CIS, or AIS) categories and analyzed the association between FGM and risk for

low grade and high grade cervical abnormalities. All analyses were conducted with Stata 14.0 (StataCorp LP, College Station, TX).

RESULTS

A total of 2,398 women ages 18-90 years old had study records with cytology and/or histology data and physical exam with indication of whether they had undergone FGM or not. Of these, 347 (14%) were diagnosed with ICC, 599 (25%) had cervical abnormalities, and 1,452 (61%) had neither ICC nor cervical abnormalities (Table 4). Just over a quarter (26%, n=633) of the study subjects had undergone FGM. Study subjects had a mean age of 38 years (SD $\pm$ 12) and had four children on average. Most study subjects had HPV (70%) and 1,085 (45%) tested positive for HIV. Close to a third of study subjects were commercial sex workers (29%). Ethnicities of study subjects included Wolof (42%), Pulaar (22%), Serere (13%), and other ethnicities (23%). Most study subjects were Muslim (88%) and the remaining were Christian, animist, or other (12%). Over half of study subjects had no formal education while 29% had primary and 18% had secondary or university education. Close to one third of study subjects reported being in a monogamous marriage, 18% reported being in a polygamous marriage, 11% reported being single, and 39% reported being either divorced, separated, or widowed. Among the 430 women in polygamous marriages, 68% had one co-wife and 32% had two or more co-wives (Table 4).

Among women with ICC, 129 (37%) had FGM, in women with cervical abnormalities, 162 (27%) had FGM, and in women negative for cervical abnormalities, 342 (24%) had FGM (Table 5). In our unadjusted model, women with ICC were 1.92 times as likely to have had undergone FGM (95% CI, 1.50-2.46). After adjusting for age, children, HIV, CSW, smoking, marital status, ethnicity, visit year, education, sex partners, and age at first pregnancy, women with ICC were 2.24 times more likely to have had FGM (95% CI, 1.12-4.49). Women with cervical abnormalities were not more likely to have had FGM in our unadjusted model (OR=1.20; 95% CI, 0.97-1.50), nor in our adjusted model (OR=1.11; 95% CI, 0.82-1.49).

In stratified analyses, adjusted for confounders, FGM remained a strong risk factor for cervical cancer (Table 6). Risk for ICC was similarly elevated (p=0.30) in both CSW (OR=2.90; 95% CI, 0.36-23.41) and non-CSW (OR=2.22; 95% CI, 1.02-4.81) with FGM, although the association was significant only amongst non-CSW as few CSW had ICC (n=6). On the other hand, CSW with cervical abnormalities were significantly more likely to have FGM (OR=2.14; CI, 1.23-3.72); however, no relationship between cervical abnormalities and FGM was observed in non-CSW (OR=0.92; CI, 0.64-1.33). In comparing the association between ICC and FGM in HIV-positive and HIV-negative women, we found that both HIV-positive and HIV-negative women with FGM were at an elevated risk for ICC. The magnitude of increased risk for ICC associated with FGM was somewhat higher in HIV-negative women (OR=2.66; 95% CI, 1.01-7.02), compared to HIV-positive women with FGM (OR=1.60; 95% CI, 0.48-5.34); this difference was borderline statistically significant (P=0.06).

In sensitivity analyses, we excluded 347 study subjects referred to Hopital Le Dantec with presumed cancer (including subjects with ICC, N=314; cervical abnormalities, N=20; and negative for cervical abnormalities, N=13) to examine whether or not the relationship between ICC and FGM differed depending on study subject recruitment method. We found that the association was no longer statistically significant (OR=1.15; 95% CI, 0.39-3.39) after restricting to the study subjects presenting to research clinics (Table 7). In other sensitivity analyses, we reclassified study subjects with ASCUS to the negative for cervical abnormalities category to evaluate the validity of our disease reclassification methods. This did not result in a substantial change of the risk estimate for ICC associated with FGM (OR=2.25; 95% CI, 1.12-4.49). Finally, we restricted to HPV-positive women to examine the likelihood of FGM in HPV-positive women with and without ICC. This analysis showed a particularly strong association in which HPV-positive women with ICC were 3.20 times more likely to have FGM (95% CI 1.29-7.96) than HPV-positive controls negative for cervical abnormalities and ICC.

Since our primary model did not show a significant association between FGM and cervical abnormalities, we sub-divided this study population group into low grade and high grade categories and examined their respective associations with FGM. We found that the likelihood of having FGM was not statistically significant in neither the women with low grade cervical abnormalities (OR=1.26; 95% CI, 0.91-1.74), nor the women with high grade cervical abnormalities (OR=0.58; 95% CI, 0.30-1.10) (Table 8).

In a final analysis we restricted to women who were negative for cervical abnormalities and ICC to examine whether HPV-positive women had a greater likelihood of exposure to FGM, to better understand the timing in which FGM may become a relevant factor in cancer pathogenesis. We found that there was no association, that HPV-positive women without cervical abnormalities and ICC were not more likely to have FGM than were HPV-negative women (OR=0.82; 95% CI, 0.55-1.24) (Table 9).

DISCUSSION

In our sample of Senegalese women with and without cervical abnormalities and invasive cancer, FGM was strongly associated with ICC. The prevalence of FGM was highest in women with ICC (37%) compared to women with cervical abnormalities (27%) and women negative for cervical abnormalities (24%). After controlling for other risk factors, we found that women with FGM were 2.24 times more likely to have ICC, a statistically significant finding (P=0.02). Additionally, this risk was significantly amplified in women who were HPV-positive with FGM. In our analyses of population subsets, the risk remained similarly elevated and statistically significant in non-CSW with FGM. HIV-positive and HIV-negative women with FGM also had elevated risk, although the magnitude of risk was somewhat greater in HIV-negative women. The difference in risk estimates for HIV-positive and HIV-negative women, which was borderline significant, warrants addition exploration to understand whether there is an unknown biological mechanism to explain this difference or confounding by unmeasured variables.

In our analysis of the relationship between FGM and cervical abnormalities in CSW, we found that CSW with FGM were at a significantly greater risk for cervical abnormalities (OR=2.14, P<0.01) compared to non-CSW with FGM (OR=0.92, P=0.65), a difference that was statistically significant (P<0.01). Given the risk of acquiring STI` is already greater in CSW, and considering that FGM results in serious scarring and inflammation of genital tissue that might make women even more vulnerable to disease and infection, it's plausible that CSW with FGM could have a greater risk of cervical abnormalities, as evidenced by these results.

Our study is the first study we know to expressly examine the association between FGM and cervical cancer. While the study conducted by Bayo et al. examined a number of risk factors in women from Mali and found women with FGM had 1.29 greater odds of having cervical cancer, our study found a substantially stronger association. Our findings suggest that FGM is a potentially important risk factor for cervical cancer disease progression and should be further evaluated in additional studies. These findings are critically important given the high prevalence of FGM in Senegal which affects a quarter of all women, and given that 1,482 women are diagnosed with cervical cancer each year, making Senegal's age-standardized cervical cancer incidence (41.4 per 100,000) one of the most elevated in sub-Saharan Africa [29]. Cervical cancer incidence and mortality could be significantly reduced with cervical cancer screening and HPV vaccination, but only 10.9% of Senegalese women aged 25-64 years old were screened in the last three years according to the 2003 World Health Survey. As reported by Bates et al., depending on the type of FGM, pelvic examination to screen for cervical cancer may be complicated or impossible due to the narrowed introitus [30]. Manji et al. describe difficulty in administering intracavitary brachytherapy to women with cervical cancer who have experienced FGM [31]. In addition to expanding HPV vaccination in sub-Saharan Africa, it is also important to educate health care providers in countries where FGM is practiced and health care providers in Western countries receiving immigrants and refugees from countries where FGM is performed so that women with FGM receive appropriate care.

Our findings should be considered in light of several limitations. Most importantly, our study was a secondary data analysis. We were therefore limited by the methods used in the parent studies to select study subjects, which could have introduced selection bias. We addressed this concern by conducting several sensitivity analyses of the relationship between FGM and cervical cancer in subsets of our study population. We found the relationship between FGM and ICC was strongest among study subjects referred with prevalent ICC. When we removed these study subjects, the risk of having ICC was not significantly greater in those with FGM (OR=1.15; 95% CI, 0.39-3.39). This suggests that much of the observed association between FGM and ICC was due to the inclusion of the population referred to the Hopital Le Dantec with presumed cancer. Since those referred with presumed cancer may be more likely to come from areas outside of Dakar while those recruited through routine screening tended to reside in the capital, it is possible that other unmeasured variables, unique to those referred with presumed cancer, could have influenced the association. For example, we did not have data on the type of FGM experienced by study subjects. It is possible that differences in FGM type, which result in varying degrees of damage to the genital tissue and in varying severity of reproductive complications, could potentially explain some of the variation in the association with cervical cancer, along with other unmeasured factors. Another limitation was that many of our study subjects, being HIV-positive and commercial sex workers, were not representative of the general population in Senegal. To address this limitation, we conducted restricted analyses in these population subsets.

Our study's strengths include its large sample size, strong methods for determining cervical disease status, and a setting which included a substantial proportion of women who had and had not undergone FGM. We also had access to rich data on the demographic and socioeconomic characteristics of study subjects, allowing for us to measure and control for various confounders of interest. In contrast, this study overcomes the limitations of the previously conducted study by Bayo et al. which had a smaller sample size (N=179) and nearly all study subjects had undergone FGM (95% cases and 93% controls). In addition, our study includes an analysis of FGM's association with non-invasive cervical abnormalities.

In conclusion, our findings suggest that advocacy efforts should continue to highlight the serious health risks and long-term complications associated with FGM. Many negative health outcomes associated with FGM are already well-documented, including urological complications and infections, complications in childbirth such as increased risk of postpartum hemorrhage, distress to the infant during delivery, and stillbirth [11]. The potential increased risk for cervical cancer, as suggested by this study, warrants further examination, especially since it is the most common cancer among women in sub-Saharan Africa. Advocacy efforts should also focus on raising awareness among health care professionals which could lead to better care and cancer-prevention for women in countries where the practice is common and for immigrant and refugee populations in Western countries.

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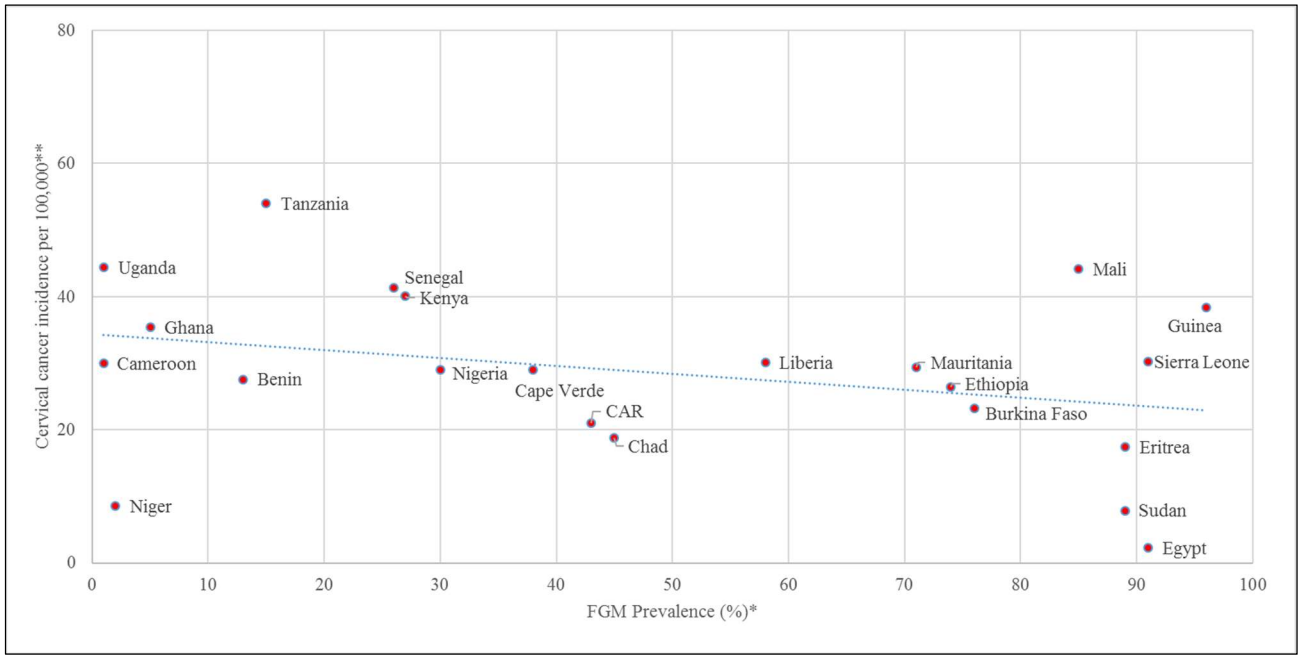
ANNEX 1

Figure 1. Relationship between FGM prevalence and cervical cancer incidence in Africa



* National prevalence of FGM by country for most recent DHS survey [22]
** 2012 IARC estimated cervical cancer rates [21]

Table 1. Outpatient study clinics and descriptions

Abbreviated Clinic Name	Description	Location
ASBEF	Family planning clinic	Dakar
Dantec	Hospital Oncology Clinic	Dakar
Fann	Outpatient Infectious Disease Clinic	Dakar
IHS	Sexually transmitted disease (STD) clinic serving commercial sex workers (CSW)	Dakar
Mbour	STD clinic serving CSW	Mbour (~62 km south of Dakar)
Pikine	Primary care clinic	Pikine (~25 km east of Dakar)
Sebik	STD clinic serving CSW	Sebikotane (~54 km east of Dakar)

Table 2. Parent studies included in the current study

No.	Study title	Screening years	Parent study	No. of subjects in current study	Age (mean)	HIV Positive
1	Natural history of cervical cancer neoplasia in HIV-1 and HIV-2	1994-1997	5392 female CSWs, adult infectious disease clinic study subjects, family planning clinic study subjects	1117	32	41%
2	Epidemiology of oral HIV infection in high-risk women	2000-2004	1052 female CSW attending STD clinics	173	38	97%
3	Control of HIV-1 by HIV-2 associated immune responses	2000-2006	2085 female CSWs, adult infectious disease clinic study subjects	233	37	93%
4	Antiretroviral therapy for HIV-2 infection in Senegal	2005-present	117 HIV 2-infected women on ART	54	49	100%
5	HIV-associated DNA hypermethylation in cervical cancer	2006-2010	1639 female study subjects	462	43	37%
6	Chlamydia and aberrant DNA methylation in cervical cancer	2007-2011	500 female study subjects	371	53	4%

Table 3. Combined histology and cytology data for disease category classification

		Histology									
		Negative	ASCUS/ Atypical	CIN1	CIN2-3	CIS/ AIS	Squamous cell carcinoma	Adeno-carcinoma	Unsat/ Missing	Other pathology	Total
Cytology	Negative	187	24	62	11	3	25	0	1,219	1	1,532
	ASCUS/Atypical	6	0	4	1	1	2	0	182	1	197
	LSIL	16	2	6	5	2	3	0	171	0	205
	HSIL	10	0	1	3	0	7	1	64	0	86
	CIS/AIS	4	0	2	3	2	9	0	23	0	43
	Squamous cell carcinoma	6	1	8	3	10	193	10	22	0	253
	Adenocarcinoma	0	0	0	0	0	10	3	1	0	14
	Unsatisfactory/missing	4	1	0	1	3	55	6	2	8	80
	Other pathology	0	0	0	0	0	0	0	0	0	0
	Total	233	28	83	27	21	304	20	1,684	10	2,410

Reclassified disease categories:

Negative for cervical abnormalities

Cervical abnormalities

ICC

Table 4. Demographic and socioeconomic characteristics of study population

	Negative for cervical abnormalities (n=1452)		Cervical abnormalities (n=599)		ICC (n=347)		Total (n=2398)	
Age, mean years ± SD	36 ± 10		37 ± 10		52 ± 13		38 ± 12	
No. of children, mean ± SD	3.5 ± 2.8		4.1 ± 2.9		6.0 ± 2.7		4.0 ± 2.9	
HPV positive (N=1949)	739	(64%)	368	(77%)	267	(84%)	1374	(70%)
HIV positive (N=2398)	722	(50%)	333	(56%)	30	(9%)	1085	(45%)
Consumes alcohol (N=2360)	210	(15%)	53	(9%)	11	(3%)	274	(12%)
Current smoker (N=2362)	317	(22%)	85	(14%)	4	(1%)	406	(17%)
CSW (N=2398)	536	(37%)	154	(26%)	6	(2%)	696	(29%)
Previously treated for STD (N=1176)	36	(4%)	13	(4%)	14	(58%)	63	(5%)
Practices vaginal cleansing (e.g, douching) (N=2001)	403	(35%)	194	(38%)	13	(4%)	610	(30%)
Birth place (N=2268)								
Senegal	1269	(94%)	542	(95%)	330	(95%)	2141	(94%)
Other West Africa	62	(5%)	23	(4%)	17	(5%)	102	(5%)
Central Africa	7	(0%)	1	(0%)	0	(0%)	8	(0%)
East Africa	2	(0%)	0	(0%)	0	(0%)	2	(0%)
Europe	1	(0%)	1	(0%)	0	(0%)	2	(0%)
Other	11	(1%)	2	(1%)	0	(0%)	13	(1%)
Ethnicity (N=2380)								
Wolof	596	(42%)	246	(41%)	143	(41%)	985	(42%)
Pulaar	308	(21%)	136	(23%)	88	(26%)	532	(22%)
Serere	182	(13%)	81	(14%)	50	(14%)	313	(13%)
Other	352	(24%)	133	(22%)	65	(19%)	550	(23%)
Religion (N=1552)								
Muslim	960	(86%)	380	(91%)	19	(90%)	1359	(88%)
Christian	145	(13%)	38	(9%)	2	(10%)	185	(12%)
Animist	0	(0%)	1	(0%)	0	(0%)	1	(0%)
Other	6	(1%)	1	(0%)	0	(0%)	7	(0%)
Education (N=2350)								
None	663	(47%)	299	(51%)	275	(80%)	1237	(53%)
Primary	457	(32%)	174	(29%)	62	(18%)	693	(29%)
Secondary/University	297	(21%)	115	(20%)	8	(2%)	420	(18%)
Marital status (N=2371)								
Single	207	(14%)	57	(10%)	5	(2%)	269	(11%)
Married monogamous	417	(29%)	199	(34%)	144	(42%)	760	(32%)
Married polygamous, 1 co-wife	150	(11%)	86	(14%)	55	(16%)	291	(12%)
Married polygamous, ≥2 co-wives	78	(5%)	32	(5%)	29	(8%)	139	(6%)
Other (divorced, separated, widowed)	583	(41%)	219	(37%)	110	(32%)	912	(39%)
Sex partners (lifetime) (N=2326)								
1	500	(35%)	232	(40%)	254	(76%)	986	(42%)
2-5	348	(25%)	176	(31%)	73	(22%)	597	(26%)
6-10	27	(2%)	13	(2%)	0	(0%)	40	(2%)
>10	541	(38%)	156	(27%)	6	(2%)	703	(30%)

Age at 1st sex (N=2109)								
10-15	493	(37%)	217	(39%)	46	(20%)	756	(36%)
16-21	660	(50%)	281	(51%)	181	(78%)	1122	(53%)
22-27	149	(11%)	45	(8%)	4	(2%)	198	(9%)
>27	24	(2%)	9	(2%)	0	(0%)	33	(2%)
Age at 1st pregnancy (N=2089)								
10-15	277	(21%)	117	(22%)	16	(7%)	410	(20%)
16-21	652	(50%)	291	(54%)	210	(87%)	1153	(55%)
22-27	203	(15%)	77	(14%)	12	(5%)	292	(14%)
>27	52	(4%)	18	(3%)	0	(0%)	70	(3%)
Never pregnant	125	(10%)	36	(7%)	3	(1%)	164	(8%)
Clinic (N=2398)								
Fann	731	(50%)	371	(62%)	21	(6%)	1123	(46%)
IHS	373	(26%)	105	(18%)	3	(1%)	481	(20%)
Mbour	142	(10%)	29	(5%)	2	(1%)	173	(7%)
Pikine	164	(11%)	69	(11%)	7	(2%)	240	(10%)
ASBEF	24	(2%)	0	(0%)	0	(0%)	24	(1%)
Sibek	5	(0%)	5	(1%)	0	(0%)	10	(1%)
Dantec	13	(1%)	20	(3%)	314	(90%)	347	(15%)
Visit year (N=2398)								
1994-2000	847	(58%)	286	(48%)	8	(2%)	1141	(47%)
2001-2006	287	(20%)	149	(25%)	10	(3%)	446	(19%)
2007-2012	318	(22%)	164	(27%)	329	(95%)	811	(34%)

Table 5. Association between FGM and invasive cervical cancer among Senegalese women

	Negative for cervical abnormalities (n=1452)		Cervical abnormalities (n=599)		ICC (n=347)	
With FGM	342	(24%)	162	(27%)	129	(37%)
Odds Ratio (OR)			1.20	(0.97-1.50)	**1.92**	**(1.50-2.46)**
OR (Adjusted)[1]			1.11	(0.82-1.49)	**2.24**	**(1.12-4.49)**

[1] model adjusts for age, children, smoking, marital status, ethnicity, visit year, education, sex partners, age at first pregnancy, CSW, and HIV

Table 6. Association between FGM and invasive cervical cancer among Senegalese women, stratified by CSW and HIV

	Negative for cervical abnormalities		Cervical abnormalities		OR (Adjusted)		ICC		OR (Adjusted)	
CSW[1]										
Not CSW with FGM	248/916	(27%)	123/445	(28%)	0.92	(0.64-1.33)	127/341	(37%)	**2.22**	**(1.02-4.81)**
CSW with FGM	94/536	(18%)	39/154	(25%)	**2.14**	**(1.23-3.72)**	2/6	(33%)	2.90	(0.36-23.41)
HIV[2]										
HIV-Negative with FGM	145/730	(20%)	67/266	(25%)	1.36	(0.85-2.18)	118/317	(37%)	**2.66**	**(1.01-7.02)**
HIV-Positive with FGM	197/722	(27%)	95/333	(29%)	0.97	(0.65-1.45)	11/30	(37%)	1.60	(0.48-5.34)

[1] Adjusted for age, children, smoking, marital status, ethnicity, visit year, education, sex partners, age at first pregnancy, and HIV

[2] Adjusted for age, children, smoking, marital status, ethnicity, visit year, education, sex partners, age at first pregnancy, and CSW

Table 7. Sensitivity analyses of the association between FGM and invasive cervical cancer in Senegalese women

	Negative for cervical abnormalities		Cervical abnormalities		OR (Adjusted[1])		ICC		OR (Adjusted[1])	
Study subjects, excluding those referred with presumed cancer										
With FGM	339/1439	(24%)	156/579	(27%)	1.10	(0.82-1.49)	11/33	(33%)	1.15	(0.39-3.39)
Study subjects with ASCUS reclassified as negative for cervical abnormalities										
With FGM	400/1662	(24%)	104/389	(27%)	1.08	(0.76-1.53)	129/347	(37%)	**2.25**	**(1.12-4.49)**
Study subjects, restricted to HPV-positive women										
With FGM	173/739	(23%)	109/368	(30%)	1.32	(0.89-1.97)	104/267	(39%)	**3.20**	**(1.29-7.96)**

[1] adjusted for age, children, smoking, marital status, ethnicity, visit year, education, sex partners, age at first pregnancy, CSW, and HIV

Table 8. Association between FGM and low and high grade cervical abnormalities in Senegalese women

	Low grade cervical abnormalities (n=464)		OR (Adjusted[1])		High grade cervical abnormalities (n=135)		OR (Adjusted[1])	
With FGM	135	(27%)	1.26	(0.91-1.74)	27	(20%)	0.58	(0.30-1.10)

[1] adjusted for age, children, smoking, marital status, ethnicity, visit year, education, sex partners, age at first pregnancy, CSW, and HIV

Table 9. Association between FGM and HPV in Senegalese women negative for cervical abnormalities

	HPV Negative (n=412)		HPV Positive (n=739)		OR		OR (Adjusted[1])	
With FGM	104	(25%)	173	(23%)	0.91	(0.68-1.20)	0.82	(0.55-1.24)

[1] Adjusted for age, children, smoking, marital status, ethnicity, visit year, education, sex partners, age at first pregnancy, CSW, and HIV