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Association of HIV with Breast Abscess and Altered Microbial Susceptibility Patterns

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ABSTRACT

Background: Breast abscesses account for 15% of surgical day cases seen in the University Teaching Hospital (UTH) in Lusaka, Zambia. Nearly all of these cases occur in lactating women. Pre-natal HIV prevalence among women seeking care at UTH was estimated at 25% as of 2004. Baseline surveys have shown that up to 60% of soft tissue infections presenting to the UTH are HIV related.

Objectives: To determine if HIV infection is a risk factor for the development of breast abscesses in women presenting to the (UTH). Secondary objective was to identify bacteriological aetiologies and drug sensitivity patterns associated with breast abscesses at UTH.

Study Design: A case-control study of 110 consecutive breast-feeding mothers diagnosed with breast abscess upon presentation to the UTH surgical service (cases) and 110 representative controls recruited from the UTH postnatal clinic.

Main Outcomes: HIV seropositivity and CD4 counts (if HIV positive) among cases and controls.

Results: Fifty-four out of 110 (49.1%) lactating women with breast abscess had positive serologic tests for HIV. Only 25 of 110 (23%) control women tested HIV positive. This difference was statistically significant, with an odds ratio of 3.28 (95% CI 1.83 – 5.87; p = 0.001). Mean CD4 counts in cases were lower than in controls (338 vs. 568, p<0.001). Staphyloccocus aureus was the main causative agent (91.8%) of isolates. Among S. aureus isolates, 70 of 101 (69.3%) were oxacillin susceptible. Forty-three of 50 (86.0%) specimens from HIV positive patients were resistant to SMX-TMP compared with only 61% of specimens from HIV negative patients (p=0.004).

Conclusions: HIV infection appears to be a significant risk factor in the development of breast abscess in lactating women in Zambia. Staphylococcus aureus remains the main causative agent, with MRSA accounting for 30.7% of isolates. SMX-TPM resistance likely stems from the wide spread use of the drug for PCP prophylaxis in HIV positive patients. It therefore should not be used for treatment of acute bacterial infections. HIV related breast infections could be considered as a possible entry point to HIV treatment now that the CD4 treatment guidelines have been adjusted to 350cells/cmm, although this requires further studies for validation.

INTRODUCTION

Lactational abscesses account for 15% of surgical infections presenting to the emergency female admission wards of the University Teaching Hospital (UTH) in Lusaka. Paucity of data on the subject, especially in sub-Saharan Africa, makes scientific statistical comparisons and

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generalisations on such a high frequency of cases difficult. A local study showed that up to 60% of patients presenting to the UTH for surgical treatment of soft tissue infections were HIV positive (Sikasote Chomba unpublished, UTH 1997).

The need for epidemiological and bacteriological data for such a widespread community acquired infection is compelling. The possible association of breast infections with HIV cannot be completely ignored as the prevalence of HIV in our general population still remains high (16%), with a higher rate in ante-natal women (25%)[1]. This is also a source of mother to child transmission of HIV. This is compounded by the fact that exclusive breast feeding for the first six months is encouraged worldwide to all mothers, especially in resource constrained areas. It is believed to reduce morbidity and mortality in infants born from infected mothers[2]. Early diagnosis and treatment of breast infections may help mitigate the increased risk of transmission associated with breast ulcerative pathologies. No studies, to our knowledge, are available that associate HIV with increased risk of breast infections in particular. However, studies have shown an increased risk of pyogenic skin infections in patients with CD4 counts below 200cells/cmm^3.

The effect of such a high disease burden in a resource limited public institution like the UTH is far reaching, from inadequate qualified surgical personnel to the cost of surgical care. Data is needed for clinical and interventional planning to help deal with the infection before it complicates beyond mastitis.

We sought to determine the HIV seroprevalence of women presenting to UTH with breast abscess and to assess if HIV infection is a risk factor for abscess development. Secondary objective was to identify bacterial and drug sensitivity patterns associated with breast abscesses at UTH and to compare these findings in HIV positive and negative women.

MATERIALS AND METHODS

A prospective case-control study was performed to evaluate the association between HIV and breast infection.

From November 2008 to February 2009, we screened consecutive patients presenting to the surgical admission ward of University Teaching Hospital with signs and symptoms of breast infection. The UTH is the only public hospital in the district with theatre capabilities and hence receives referrals for all complicated and many uncomplicated cases of mastitis requiring surgical intervention.

Breast abscess was defined as a fluctuant inflammatory tender breast mass with positive needle aspiration of pus. Patients were included as cases if they had a confirmed diagnosis of breast abscess, were lactating, and lived in Lusaka district. Patients were excluded if they had a previous diagnosis of diabes or resided outside of Lusaka district. The control group consisted of breast feeding women attending antenatal clinic at UTH. The case and control groups were frequency matched to control for patient age, education level, and parity. Written informed consent was obtained from all case and control patients. The study was approved by the University of Zambia Research Ethics Committee.

Epidemiological data was collected by a standard questionnaire applied to both sets of clients.

The following specimens were collected:

1. Blood for HIV antibody testing, and reflexive CD4 count if HIV test was positive
2. Pus Aspirates for microscopy, culture, and sensitivity.

Specimens were analyzed at the UTH virology labs. HIV tests were performed using the Determine® HIV-1/2 antibody test (Inverness Medical Innovations, Cranfield, UK) for screening and Uni-Gold Recombigen® HIV Test (Trinity BioTech, Bray, Ireland) for confirmation. CD4 counts were done by FACS calibur machine and blood was stained using CD3 FITC, CD4 PE and CD45 PERCP monoclonal Abs. Pus aspirates were cultured on Mueller-Hinton agar. We tested drug susceptibilities of standard antibiotics currently recommended by government for use in public clinics for first and second line management of infections. Kirby-Bauer disk diffusion method was

used, and MIC resistance cutoffs were obtained from the Clinical and Laboratory Standards Institute Quality Manual, 2008 edition (CLSI, Wayne, PA, USA).

Using EPI-INFO 6 Stat calc (Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA), we estimated that we would need a sample size of 375 total patients in order to achieve an 80% power to detect an odds ratio of 2.0 or more with an alpha of 0.05.

In our pre-study survey, a total of 236 cases of breast abscess were seen in seven months. Based on time constraints, however, we limited our study to 110 case patients meeting inclusion criteria over the course of 3months and 110 controls.

Characteristics of cases and controls were compared using Chi squared test or Fisher's exact test for categorical variables and student's t test for continuous variables. All variables with a p value of < 0.1 were included in a logistic regression model to further assess association with abscess. Analyses were performed using SPSS Version17 (SPSS, Inc., Chicago, IL, USA). P value of less than 0.05 was considered statistically significant.

RESULTS

One hundred ten cases of breast abscess were identified along with one hundred ten postnatal controls. Baseline characteristics of cases and controls are shown in Table 1. Patients ranged in age from 16 to 34 with an average age of 23 years. Eighty-six percent were married. The majority of women lived in high-density neighbourhoods (74%), were employed (63%) and had only one child (53%). Eighty percent of women had a child less than six months of age. Twenty-five percent of patients endorsed drinking alcohol, but only 3% of patients readily admitted to smoking cigarettes. There was a trend towards increased alcohol use among case patients, although not statistically significant (OR 1.82, 95%CI 0.97-3.40).

Table 1: Baseline characteristics of cases and controls

	Cases n=110	Controls n=110	P
Age, years (SD)	23.0 (4.4)	22.4 (4.1)	0.31
Married, no. (%)	94 (86)	96 (87)	0.92
Parity, no. (%)			0.054
1	62 (56)	54 (49)	
2	26 (24)	45 (41)	
3 or more	22 (20)	11 (10)	
Residence, no. (%)			0.35
High-density	84 (76)	78 (71)	
Medium-/Low-density	26 (24)	32 (29)	
Education level, no. (%)			0.79
None or primary	52 (47)	54 (49)	
Secondary or higher	58 (53)	56 (51)	
Age of child, no. (%)			0.15
0-6 months	90 (82)	85 (77)	
>6 months	16 (18)	25 (23)	
Cigarette smoking, no. (%)	4 (3.6)	3 (2.7)	0.70
Alcohol use, no. (%)	33 (30)	21 (19)	0.06
Prior breast infection, no. (%)	15 (13.6)	9 (8.2)	0.19
HIV seropositive, no (%)	54 (49)	25 (23)	0.001
CD4 count (if HIV+),cells/mm^3 (SD)	338 (160)	568 (123)	<0.001

Primary outcomes

Fifty-four out of 110 (49.1%) lactating women with breast abscess had positive serologic tests for HIV. Only 24 of 110 (21.8%) control women tested HIV positive. This difference was statistically significant, with an odds ratio of 3.46 (95% CI 1.92 – 6.21; p = 0.001). In a multivariate logistic regression analysis that included all of the variables in Table 1 except for CD4 count, HIV status remained significant (OR 3.20, 95%CI 1.71 – 5.98). Mean CD4 counts among HIV positive women with breast abscess was 338cells/mm^3 (range: 59 to 850) and for HIV positive controls were 568 cells/cmm (range: 420 to 712; p<0.001). Forty-one out of 54 (76%) HIV positive case patients had CD4 counts between 201 and 500. Only six (11%) had CD4 at or below 200, and the remaining seven patients (13%) had CD4 counts greater than 500. None of the control patients had CD4 counts less than 200.

Secondary outcomes

Microbiologic results

Pus aspirates were obtained from all 110 cases and were cultured for identification of organisms. Drug susceptibility testing was performed for oxacillin and for commonly available antibiotics. Results were unavailable for two specimens. Of the

remaining 108 samples, 101 (91.8%) grew *Staphylococcus aureus*, one sample (0.9%) grew bacillus species and one sample (0.9%) grew *Streptococcus pyogenes*. Five samples (4.5%) were culture negative.

Among *S. aureus* isolates, 70 of 101 (69.3%) were oxacillin susceptible, while the remaining 31 (30.7%) were oxacillin-resistant (MRSA). Twenty-seven (26.7%) and fifty-three (52.5%) isolates were susceptible to sulfamethoxazole-trimethoprim (SMX-TMP) and tetracycline, respectively. Chloramphenicol (93%), erythromycin (95%), and ciprofloxacin (99%) had in vitro activity against more than ninety percent of isolates.

Figure 1

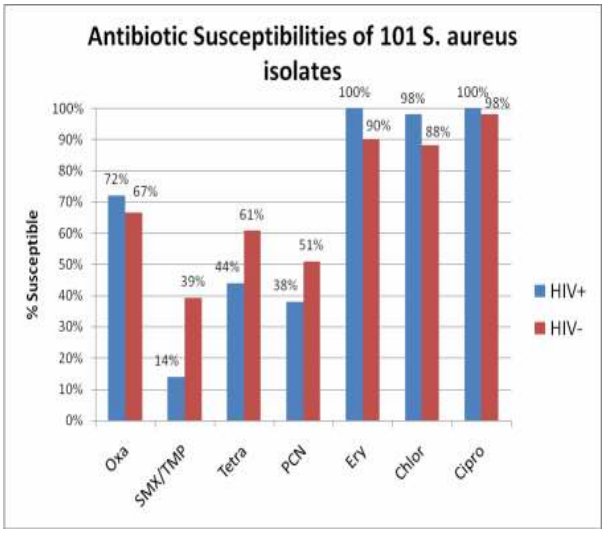

Figure 1 shows S. aureus drug susceptibilities by HIV serostatus. Forty-three of 50 (86.0%) specimens from HIV positive patients were resistant to SMX-TMP compared with only 61% of HIV negative patients (p=0.004). There were no other significant differences in drug susceptibilities between HIV positive and negative patient isolates.

DISCUSSION

International literature shows mastitis to be common between 5%-33%, with complication rates between 1-3% [4-7]. Most complications have been attributed to delayed treatment of mastitis and not HIV immunosuppression. Although recurrent pyogenic skin infections are a known feature of severe immunosuppression (CD4>200cells/cmm), there are no studies that directly ascribe development of mastitis and breast abscesses to HIV infection[3].

In our study we found a significant association between HIV infection and development of breast infections (Odds ratio 3.46). This association was not affected by other measured variables in a logistic regression model (OR 3.77). We also found lower mean CD4 counts amongst HIV+ cases compared with HIV +controls. The majority of HIV positive patients with breast abscess had CD4 counts between 200 and 500. Our findings demonstrate that mild to moderate immunosuppression predisposes HIV positive patients to soft tissue infections. Patients do not need to progress to AIDS in order to be at risk for breast abscess. Only 6 patients with abscesses had CD4 counts (< 200) that would qualify for AIDS.

Several risk factors have already been identified to be associated with the development of mastitis. These include cigarette smoking(in non lactating women), anaemia, diabetes, use of steroids, fatigue, low socio-economic status, state of reduced immunity not specified as HIV infection, postpartum pelvic infection[8-10]. In our study cigarette smoking was not significantly associated with mastitis (p=0.70). Smoking is culturally not a common habit in women in Zambia. Alcohol consumption, however, is somewhat common, and we found a trend toward association between alcohol use and abscess. Age of breast feeding child (0-6months) was also not a significant factor although observed internationally[4]. Most cases seen were in employment which may be a source of mother fatigue.

The predominance of *Staphyloccocus aureus* as a cause of breast abscesses in this study was consistent with previous studies that examined breast infections [11, 12]. Whereas those studies described women with both complicated and uncomplicated mastitis, our study included only those with breast abscess.

The proportion of *S. aureus* isolates in our study that were MRSA by oxacillin testing (30.7%) was

comparable with studies performed in South Africa[13]. To our knowledge, ours is the first published study to describe rates of MRSA infection in Zambia. When there is minimal cellulitis surrounding a breast abscess, surgical management without antibiotics is generally sufficient. For cellulitis of the breast and other areas of the body, however, treatment usually consists of cloxacillin or cephalexin. Our findings suggest that in soft tissue infections complicated by abscess formation, if antibiotics are required they should include MRSA coverage, particularly in severe or life-threatening infections.

The choice of antibiotic for MRSA coverage is complicated. In other parts of the world, SMX-TMP is used frequently for community-acquired MRSA treatment. In the United States, for example, studies have found that 95-99% of methicillin-susceptible and -resistant *S. aureus* strains were SMX-TMP susceptible[14,15]. Our study found very high rates (73.3%) of SMX-TMP resistance, with even higher resistance rates (86.0%) seen in HIV positive patients. This finding reflects the widespread use of the drug in HIV positive patients for the prophylaxis of pneumocystis, toxoplasma, and bacterial infections. WHO guidelines recommend SMX-TMP for all HIV positive patients with stage 2 disease or higher regardless of CD4 count. This has been an effective strategy, but its impact on resistance profiles needs to be considered when empirically treating bacterial infections.

Consequently, drugs other than SMX-TMP should be used for treating *S. aureus* or suspected *S. aureus*. Our study found that staph isolates were most likely to be susceptible in vitro to chloramphenicol, erythromycin, or ciprofloxacin. The high susceptibilities to these drugs are contrary to findings from Great Britain and the U.S.[14,16]. Staph aureus isolates from nearby South Africa have shown variable resistance patterns[13,17]. In KwaZulu-Natal province, for example, overall rates of resistance were 30% for erythromycin and 5% for chloramphenicol and ciprofloxacin, but much higher in the methicillin-resistant strains. Nationwide, 70-80% of MRSA strains were resistant to erythromycin or ciprofloxacin[17]. The extremely low rates of resistance to these drugs in our study might reflect a difference in regional antimicrobial usage and subsequent epidemiology. Erythromycin, ciprofloxacin, and chloramphenicol should be considered, alone or in combination for treatment of suspected staphylococcal soft tissue infections. Clindamycin, although not assessed in this study, is another possible alternative.

There were several limitations in our study. As in any case-control study, the selection of controls has a strong bearing on the results. The CD4 counts among our controls were higher than might be expected from our general population. This may reflect the fact that women with lower CD4 counts may have been diagnosed during pregnancy and received ART for some duration. We did not assess the use of antiretrovirals among our cases or control patients. Although our case patients were referred from throughout Lusaka, our controls were patients who specifically receive postnatal care at UTH and might not be representative of the neighbourhoods where case patients live. However, the proportion of patients from high-density neighbourhoods was similar in the two groups.

CONCLUSION

HIV infection appears to be a significant risk factor in the development of breast infections in lactating women presenting to the UTH (Lusaka). *Staphylococcus aureus* remains the main causative agent with MRSA accounting for (30.7%) of isolates. Sulfamethoxazole-trimethoprim resistance (74.3%) likely stems from the wide spread use of the drug for PCP prophylaxis in HIV positive patients. It therefore should not be used for treatment of acute bacterial infections. All women in Zambia with lactational breast abscess should be tested for HIV. HIV-related breast infections could be considered as a possible entry point to HIV treatment now that the CD4 treatment guidelines have been adjusted to 350cells/cmm, although this requires further studies for validation.

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REFERENCES

1. Zambia Ministry of Health, Central Board of Health. Zambia Antenatal Clinic Sentinel Surveillance Report: 1994-2004. Lusaka: Zambia Ministry of Health, Central Board of Health; 2005.
2. Coovadia H.M. et al, Mother to child transmission of HIV-1 infection during exclusive Breast Feeding; the first six months of life. *The Lancet* 31 mar 2007 Vol 369 issue 9567, P1107-1116
3. CDC Classification System for HIV-Infected Adults and Adolescents
4. Allison Vogel, B,L Hutchison, E,A Mitchell Mastitis in the first year post partum *Birth Vol* 26 (4), 218-225.
5. Foxman B, Dancy H, Gillespie B, Lactation Mastitis; occurance and medical management among 946 breastfeeding women in the US. *AMJ Epidemiology* 2002; 155:103-114
6. Jonsson S, Pulkkinen M O. Mastitis today,incidence, prevention and treatment, *Ann Chiurg Gynaecolog* 1994:33;84-87
7. Amir Lisa H, Foster Della, Mclachlan Helle, Lumly Judith. Incidence of breast abscess in lactating women, a report from an Australian cohort. *BJOG;* 2004, 111; 12 , 1378-1381.
8. Kinlay J R,O`Connell D Z,Kinlay S. Risk factors for mastitis in Breastfeeding females, Results of a prospective cohort study. *Aust NZJ public Health* 2001:25;115-120
9. Dener C, Inan t, et al, Breast Abscess in lactational women. *World Journal of Surgery* 2003 Feb, 27(2):130-3 Efem SE. Breast Abscesses in Nigeria. Lactational Vs Nonlactational. *J R Coll Surg Edinb*. 1995-Feb; 40(1): 25-7.
10. Cibeleli b, Kendra Shwarz, Belsy Foxman. Lactation Mastitis; *JAMA*, 2003, 289 (13).
11. J.M, Dixon, ABC of Breast infection. *BMJ* 1994; 309:946-949 (8) October.
12. WHO. Mastitis causes and management. Geneva dept of child and adolescent health development 2000
13. Shittu, A.O. and Lin, J. Antimicrobial susceptibility patterns and characterization of clinical isolates of Staphylococcus aureus in KwaZulu-Natal province, South Africa. *BMC Infectious Diseases* 2006; 6:125.
14. Mendes, R.E., Sader, H.S., Deshpande, L.M., et al. Characterization of baseline methicillin-resistant Staphylococcus aureus isolates recovered from phase IV clinical trial for linezolid. *Journal of Clinical Microbiology* 2010; 48:568-574
15. Draghi, D.C., Sheehan, D.F., Hogan, P., et al. Current antimicrobial resistance profiles among methicillin-resistant Staphylococcus aureus encountered in the outpatient setting. *Diagnostic Microbiology and Infectious Disease* 2006; 55:129-133.
16. Gemmell, C.G., Edwards, D.I., Fraise, A.P., et al. Guidelines for the prophylaxis and treatment of methicillin-resistant Staphylococcus aureus (MRSA) infections in the UK. *Journal of Antimicrobial Chemotherapy* 2006; 57:589-608.
17. Marais, E., Aithma, N., Perovic, O., et al. Antimicrobial susceptibility of methicillin-resistant Staphylococcus aureus isolates from South Africa. *South African Medical Journal* 2009; 99:170-173.