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Risk factors associated with low CD4+ lymphocyte count among HIV-positive pregnant women in Nigeria

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Abstract

Objective—To determine the risk factors for CD4+ lymphocyte counts of 200 cells/mm³ or lower in HIV-positive pregnant women in Nigeria.

Method—A cross-sectional data analysis from a prospective cohort of 515 HIV-positive women attending a prenatal clinic. Risk of a low CD4+ count was estimated using logistic regression analysis.

Results—CD4+ lymphocyte counts of 200 cells/mm³ or lower (280±182 cells/mm³) were recorded in 187 (36.3%) out of 515 HIV-positive pregnant women included in the study. Low CD4+ count was associated with older age (adjusted odds ratio [aOR] 10.71; 95% confidence interval [CI], 1.20–95.53), lack of condom use (aOR, 5.16; 95% CI, 1.12–23.8), history of genital ulcers (aOR, 1.78; 95% CI, 1.12–2.82), and history of vaginal discharge (aOR; 1.62; 1.06–2.48).

Conclusions—Over 35% of the HIV-positive pregnant women had low CD4+ counts, indicating the need for treatment. The findings underscore the need to integrate prevention of mother-to-child transmission with HIV treatment and care, particularly services for sexually transmitted infections.

Keywords

Africa; HIV; Low CD4+ lymphocyte count; Prevention of mother-to-child transmission; HIV-positive pregnant women

Introduction

Since 2005, international and local efforts have resulted in increased access to antiretroviral treatment for HIV-positive Nigerians; more than 120 000 patients are receiving therapy compared with approximately 10 000 in 2003 [1]. Access to high-quality prevention of mother to child transmission (PMTCT) services has increased [2], but coverage remains low at 5.25%, and the linkage between PMTCT and other available HIV services needs to be strengthened [1].

HIV-positive pregnant women need a wide range of HIV services, and prenatal clinics are key access points for prevention of transmission and provision of antiretroviral therapy services where mothers, their infants, and their partners can be effectively targeted for HIV interventions [3–5]. Further scale-up of prevention and treatment services in low-resource countries will require the integration of PMTCT programs with antiretroviral therapy and other services.

Identifying the predictors of a low CD4+ lymphocyte count in resource-limited settings can help guide the appropriate use of prophylactic interventions, help target treatment to populations at greatest risk of HIV transmission, and serve as an immediate bridge between PMTCT and other services, especially in the absence of CD4 technology. The CD4+ lymphocyte count is a valid independent predictor of disease stage and correlates inversely with disease progression [6–8]. Increased risk of HIV transmission from mother to child in the perinatal period is associated with a low maternal CD4+ count and provides further support for the importance of this indicator in the management of HIV-positive pregnant women [5, 9].

The aim of the present study was to determine the risk factors associated with a CD4+ lymphocyte count of 200 cells/mm³ or lower, an indication for antiretroviral therapy, by conducting a cross-sectional data analysis from a prospective cohort established to monitor and evaluate the implementation of a PMTCT program.

Materials and methods

Between April 2004 and March 2006, 13 032 pregnant women were screened for HIV-1 at Plateau State Specialist Hospital (PSSH) prenatal clinic in Jos, Nigeria. All women attending the prenatal clinics received general counseling regarding health and HIV prevention. Women opting for HIV testing were counseled individually before and after the test. Free HIV testing was provided regardless of the decision to participate in the study. Of the 593 HIV-positive pregnant women, 515 (86.8%) participated in the study. CD4+ lymphocyte counts were taken at enrollment and/or at delivery. Prior to enrollment in the study, written informed consent was obtained from patients, and approval was obtained from the University of Maryland Institutional Review Board and the PSSH Ethics Committee.

At enrollment, a detailed medical history was taken, a physical examination was performed, and a 10-mL blood sample was obtained by a trained physician. Demographic information and nonclinical data on patients were obtained from questionnaires completed during interviews with nurse counselors.

HIV seropositivity was defined as: (1) positive results on 2 different rapid HIV tests conducted sequentially (Uni-Gold [Trinity Biotech, Bray, Ireland] and Determine HIV-1/2 test [Inverness Medical, UK]); or (2) an initial positive enzyme-linked immunosorbent assay (ELISA; Vironostika, BioMerieux, France) antibody test followed by a positive HIV rapid test; or (3) a positive western blot (QualiCode HIV-1/2 Kit; Immunitics, Boston, USA) if there was discordance in the preceding 2 testing methods. Blood samples collected at the first prenatal visit and/or at the time of delivery were used for determining CD4+ lymphocyte counts (FACSCount System; Becton-Dickinson, Franklin Lakes, NJ, USA). Vaginal infections were detected using wet mounts and microscopy to indicate the presence of clue cells, indicating bacterial vaginosis; motile *Trichomonas vaginalis*; and yeast cells indicating candidiasis. Bacterial vaginosis was diagnosed according to the Amsel diagnostic criteria. Syphilis data were added mid-way through recruitment and were based on a rapid plasma regain test (Reagin Screen Test Kit, Arlington Scientific, Springville, UT, USA) and positive confirmation was by *Treponema pallidum* hemagglutination assay (Arlington Scientific).

The CD4+ lymphocyte count was determined as less than or equal to 200 cells/mm³ (low CD4+ count) or more than 200 cells/mm³ based on the current national treatment criteria and the WHO guidelines [10]. History of sexually transmitted infections (STIs) and symptoms in the last year were assessed as categorical variables (Yes, No, or Don't Know). Univariate analyses were conducted to determine variables associated with a low CD4+ count using the χ^2 test for categorical variables and the t test for continuous variables.

Logistic regression models were fitted using known risk factors for low CD4+ count and variables associated with low CD4 count in the univariate analysis at $P \leq 0.05$. A multivariate model was fitted, and potential interaction was assessed by including interaction terms in the regression equation or by stratification. Potential confounders were adjusted for in the multivariate analysis and final model. Test of homogeneity of stratified estimates or Wald statistic were used to assess heterogeneity. On the basis of the assessment of the Hosmer-Lemeshow Goodness-of-Fit test and the Akaike's Information Criterion (AIC), the final most parsimonious model, with an $R^2=0.107$ and C statistic=0.698, consisted of the variables: age, religion, occupation, parity, knowledge of husband's/partner's HIV status, condom use, history of STI, and genital discharge or ulcer in the last 12 months. Certain variables significant at the 0.1 level were retained in the model and improved the overall fit. Data analysis was conducted using SAS Release 8.00 (SAS Institute, Cary, NC, USA).

Results

Sociodemographic characteristics of the 515 study participants are presented in Table 1. There were no differences in age, education, religion, age at first sexual intercourse, and marital status between the women who agreed to participate in the study and those who did not. The mean age of the participants was 26.8 ± 4.9 years (range, 15–45 years); most were married and Christian (92.4% and 81.2%, respectively). Most (82.9%) did not know their husband's/partner's HIV status; most (96.6%) exhibited characteristics and behaviors that could increase the risk of HIV transmission, such as lack of consistent condom use and at least one laboratory-confirmed STI. A total of 130 (25.2%) women reported having had a genital ulcer in the past 12 months, and 128 (24.8%) reported a history of one STI in the past year (Table 2). Most of the 259 (50.3%) women who had a laboratory confirmed vaginal infection had bacterial vaginosis or candidiasis (48.7% and 46.3%, respectively). Overall, 283 (56.3%) women had had 2 or more sexual partners in the last 5 years, and 289 (60.6%) reported not using condoms with any of their sexual partners.

CD4+ lymphocyte counts of 200 cells/mm³ or lower (280 ± 182 cells/mm³) were recorded in 187 (36.3%) of the 515 HIV-positive pregnant women in the study. Analyses to determine any associations between the sociodemographic variables and low CD4+ count (Table 2) showed that age, parity, and history of vaginal ulcer, STI, or vaginal discharge in the last year were associated with a low CD4+ count. Compared with individuals who had a CD4+ count of more than 200 cells/mm³, women with low CD4+ counts were more likely to be older ($P=0.018$ for trend; adjusted odds ratio [aOR] 10.71; 95% confidence interval [CI], 1.20–95.53). The proportion of women with a low CD4+ count increased with parity ($P=0.005$ for trend). Interestingly, although not significant, Muslim women were 1.6 times more likely to have a low CD4+ count compared with Christian women ($P=0.064$).

Self-reporting of and symptoms of STIs were associated with a low CD4+ count. Women reporting a history of STI in the last year had lower CD4 counts compared with women who reported no history of STIs (45.3% vs 32.1%, respectively, $P=0.024$). Women who reported having a genital ulcer in the last year were more likely to have lower CD4+ counts (odds ratio [OR] 1.8; 95% CI, 1.20–2.70). Having an abnormal discharge in the last year was also associated with a low CD4+ count (42.0% vs 28.6% with no history of discharge, $P=0.006$) as was the number of symptoms indicative of an STI, with increasing association as the number of symptoms increased ($P=0.001$). There was no association between the number of sexual partners in the last 5 years and a low CD4+ count.

There was a significant association between frequency of condom use in the last 12 months and low CD4+ count. Compared with women who reported consistent condom use, women who reported no condom use were 5 times more likely to have low CD4+ counts (OR 4.63;

95% CI, 1.03–20.7). Although an increased association with low CD4+ counts was observed for women who did not use condoms frequently (OR 3.12; 95% CI, 0.68–142), the association was not significant.

We constructed a logistic regression to delineate characteristics associated with low CD4+ counts (Table 3). Maternal age (OR 6.88 for 25–29 years of age and OR 7.48 for >30 years of age), religion (OR 1.70 for Muslim women), parity frequency of condom use (OR 5.16 for no condom use), and the self-reported history of STIs (OR 1.62 for abnormal vaginal discharge and OR 1.78 for a genital ulcer in the last year) remained independently and strongly correlated with low CD4+ counts.

Discussion

The key findings of the present study were that most high-risk factors for HIV—including not using condoms, past history of genital ulcers, a previous history of STIs, and older age—were significant predictors of low CD4+ count in this prenatal population, which underscores the need to integrate antiretroviral therapy services and other prevention services for HIV into PMTCT programs. Over one third of the women enrolled in the study were eligible for antiretroviral therapy based on their immunologic status. New evidence is now emerging indicating that the risk of complications of HIV increases if treatment is delayed and provides support for starting treatment at CD4+ levels of 350 cells/mm³ or even higher, especially for certain subgroups of HIV-infected patients including pregnant women [11]. If Nigeria were to revise its treatment guidelines to optimize clinical outcomes based on evidence-based data, almost 75% of its prenatal population would be eligible for antiretroviral therapy.

Age has been demonstrated to be a predictor of low CD4+ count in both HIV-positive and HIV-negative populations. A study conducted among HIV-negative pregnant women in the same population also found an association between older age and lower CD4+ counts (b350 cells/mm³), although the findings among the HIV-positive women in this analysis were more strongly associated with more narrowly defined age categories [12]. Older women may have been infected with HIV for longer and, therefore, have a lower CD4+ count because of disease progression. Older age at the time of HIV infection has been found to be associated with a high early viral load and a more rapid disease progression [13]. It has also been shown that with aging, the immune system declines, specifically with regards to the level of CD4+ T lymphocytes, although nutritional status may play a role [14,15].

There was a high prevalence of vaginal infections in the study population, and more than half of the women had at least one laboratory-confirmed infection. Further investigation of the prevalence of these infections in the study population showed that many infections were due to bacterial vaginosis and candidiasis. The evidence of how these infections may affect HIV transmission and acquisition is mounting [16,17]. The prevalence of these vaginal infections, particularly bacterial vaginosis, is high in many Sub-Saharan African countries and is generally higher than ulcerative STIs among pregnant women. For example, prevalence estimated from studies of prenatal clinic attendees was 52% in South Africa [18] and 24% in Tanzania [19]. Between 20% and 50% of women of reproductive age in Zimbabwe [20] and Uganda [21] are affected by bacterial vaginosis. These vaginal infections are generally not diagnosed in a laboratory and are not treated during prenatal care. Further research is needed to characterize the occurrence of these infections in relation to pregnancy and to determine how they affect pregnancy outcomes.

Although our previous studies in this population documented an association of self-reported history of STI, genital ulcer, or vaginal discharge with risk of acquiring HIV (unpublished data), we were surprised to find these factors among the strongest factors associated with a low

CD4+ count. A woman who reported an STI in the previous 12 months had a 1.7-fold chance of having a low CD4+ count compared with a woman who reported no STIs. Other markers of STIs, such as history of genital ulcer and vaginal discharge, confirmed the association of STIs with a low CD4+ count, but this may also be related to reactivation of latent infections. Herpes simplex virus 2 (HSV-2) is one of the more common causes of genital ulcerative disease in Africa; several studies show biological synergy between HSV-2 and HIV infection, resulting in an increased HIV viral load and a cyclical effect of HSV-2 reactivation and HIV replication [22–24]. A recent study by Duffus et al. [25] found that chronic HSV-2 infection was not associated with low CD4+ count; the authors suggest that the observed increase in HIV viral load could precede a decrease in CD4+ cell count, but better-powered studies are warranted to investigate this relationship further. This is clearly an area that requires further investigation as it has the potential for high public health impact.

The low level of condom use and the association of lack of condom use with low CD4+ cell count are of concern because of the implications for HIV transmission to uninfected partners. Efforts to prevent the transmission of HIV are essential to curb the HIV/AIDS epidemic, and our findings suggest that prenatal clinics could serve as a good venue for scaling up prevention with positive activities. Overall, 82.9% of the women did not know the HIV status of their husband/partner; this was even more pronounced for women with a low CD4+ count. This finding demonstrates a lack of disclosure in a population that is sexually active, and the potential of increasing the spread of HIV because of high-risk factors, such as low condom use, presence of STI, and a potentially high viral load. Prevention programs in this population must, therefore, enhance counseling of couples linked to treatment and care programs for the entire family unit.

The observed association of Muslim religion with low CD4+ count is an interesting finding. This could be because Muslim women are less likely to come to a prenatal clinic at a large specialist hospital until they are very ill. A majority of Muslim women enrolled in the study were recruited from satellite clinics that offer only primary care, an indicator of lack of access to secondary and tertiary care. Often in this setting, the men are the dominant decision-makers for the women and children, which impacts when and what kind of care HIV-positive women and children can access. Targeting culturally sensitive interventions that incorporate the husband/partner into prevention and treatment programs could have significant long-term benefits in this population, in which over 90% of the women attending prenatal clinics are married.

There were some limitations to the study. As in many studies, there is the potential for selection/enrollment bias. Pregnant women coming to the health facility may be in poor health and more likely to have characteristics that increase the risk for low CD4+ count. In addition, STI data were self-reported and were not confirmed by laboratory and clinical testing. Furthermore, because the study was based on monitoring and evaluating a PMTCT program, there were no data on time since HIV infection, which is known to be associated with a decline in CD4+ count. Therefore, confounding due to the duration of infection could not be investigated. Nonetheless, the study provides valuable information on the CD4+ status of HIV-positive pregnant women and risk factors of this population, in addition to defining characteristics of a population in need of treatment and other wraparound services.

Increased access to treatment has the potential to alleviate some of the burden of HIV/AIDS in Nigeria, but the process will be hindered unless the current infrastructure is effectively utilized and built upon. As Nigeria makes choices about expanding treatment for HIV/AIDS, the findings of the present study illustrate the needs in the prenatal setting. With more than 1 in 3 women attending the prenatal clinic requiring treatment, PMTCT programs appear to be a logical and effective way to roll out antiretroviral therapy and to link treatment with other

services. Establishing antiretroviral sites where PMTCT sites already exist is an effective way to use the infrastructure already in place.

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Table 1Sociodemographic characteristics of the study population (n = 515). ^a

Characteristics	No.	(%)
Age, y		
15–19	24	(4.7)
20–24	143	(28.2)
25–29	213	(42.0)
≥ 30	127	(25.1)
Age at marriage, y		
12–16	54	(11.0)
17–21	192	(39.3)
22–26	197	(40.3)
≥ 27	46	(9.4)
Highest education completed		
Lower than high school	299	(58.6)
High school or above	211	(41.4)
Marital status		
Married	471	(92.4)
Unmarried	39	(7.6)
Religion		
Christian ^b	414	(81.2)
Muslim	85	(16.7)
Other	11	(2.16)
Occupation		
Student	29	(5.7)
Unemployed	202	(39.6)
Employed	279	(54.7)
Number of pregnancies		
1	123	(23.9)
2	112	(21.8)
3	108	(21.0)
≥ 4	171	(33.3)
Age at first sexual intercourse, y		
≤ 14	25	(4.9)
15–19	276	(54.4)
20–24	144	(28.4)
≥ 25	62	(12.2)
Aware of husband's/partner's HIV status		
No	402	(82.9)

Characteristics	No.	(%)
Yes	83	(17.1)

^aNumbers do not always total 515 because of missing data.

^bDefined as Catholic, Pentecostal, or Protestant.

Table 2

Characteristics associated with CD4+ lymphocyte count of less than or equal to 200 cells/mm³ in the study participants (n = 515).^a

Characteristics	Total no. of women	Women with a CD4+ count ≤ 200 cells/mm ³		OR (95% CI)	P value ^b
		No.	(%)		
Age, y					
15-19	24	4	(16.7)	Referent	0.018 ^c
20-24	143	43	(30.1)	2.15 (0.69-6.66)	
25-29	213	81	(38.0)	3.07 (1.01-9.30)	
≥ 30	127	56	(44.1)	3.94 (1.27-12.20)	
Age at marriage, y					
12-16	54	23	(42.6)	Referent	0.594
17-21	192	71	(37.0)	0.79 (0.43-1.46)	
22-26	197	65	(32.3)	0.66 (0.36-1.23)	
≥ 27	46	16	(34.8)	0.72 (0.32-1.62)	
Education, y					
0-11	299	111	(37.1)	Referent	0.558
≥ 12	211	73	(34.6)	0.90 (0.62-1.29)	
Marital status					
Married	471	172	(36.5)	Referent	0.473
Unmarried	39	12	(30.8)	0.77 (0.38-1.56)	
Religion					
Christian	414	143	(34.5)	Referent	0.064
Muslim	85	39	(45.9)	1.61 (1.00-2.58)	
Other	11	2	(18.2)	0.42 (0.09-1.97)	
Occupation					
Student	29	7	(24.1)	Referent	0.077
Unemployed	202	65	(32.2)	1.49 (0.61-3.67)	
Employed	279	112	(40.1)	2.11 (0.87-5.10)	
Number of pregnancies					

Characteristics	Total no. of women	Women with a CD4+ count ≤ 200 cells/mm ³		OR (95% CI)	P value ^b
		No.	(%)		
1	123	42	(34.1)	Referent	0.005 ^c
2	112	27	(24.1)	0.61 (0.35–1.08)	
3	108	42	(38.9)	1.23 (0.72–2.10)	
≥ 4	171	76	(44.4)	1.54 (0.96–2.49)	
Age at first sexual intercourse, y					
≤ 14	25	7	(28.0)	Referent	0.768
15–19	276	101	(36.6)	1.48 (0.60–3.67)	
20–24	144	53	(36.8)	1.50 (0.59–3.82)	
≥ 25	62	20	(32.3)	1.22 (0.44–3.40)	
Aware of husband's/partner's HIV status					
No	402	150	(37.3)	Referent	0.214
Yes	83	25	(30.12)	0.72 (0.43–1.21)	
Genital ulcer in the last 12 months					
No	383	126	(32.9)	Referent	0.004 ^c
Yes	130	61	(46.9)	1.80 (1.20–2.70)	
STI in the last 12 months					
No	324	104	(32.1)	Referent	0.024 ^c
Yes	128	58	(45.3)	1.75 (1.15–2.66)	
Don't know	59	24	(40.7)	1.45 (0.82–2.56)	
Vaginal discharge in the last 12 months					
No	213	61	(28.6)	Referent	0.006 ^c
Yes	295	124	(42.0)	1.81 (1.24–2.63)	
Don't know	1	0	(0)	0	
No. of symptoms of STI in the last 12 months					
None	168	47	(28.0)	Referent	0.001 ^c
1	172	58	(33.7)	1.31 (0.83–2.08)	
2	120	53	(44.2)	2.04 (1.24–3.34)	
3	45	26	(57.8)	3.52 (1.78–6.96)	

Characteristics	Total no. of women	Women with a CD4+ count ≤ 200 cells/mm ³		OR (95% CI)	P value ^b
		No.	(%)		
Husband/partner has multiple sex partners					
No	288	98	(34.0)	Referent	0.438
Yes	102	36	(35.3)	1.05 (0.66–1.70)	
Don't know	97	40	(41.2)	1.36 (0.85–2.18)	
Number of sexual partners in the last 5 years					
0–1	220	82	(37.3)	Referent	0.600
2	141	52	(36.9)	0.98 (0.63–1.52)	
3	77	23	(29.9)	0.72 (0.41–1.25)	
≥ 4	65	26	(40.0)	1.12 (0.64–1.98)	
Condom use in the last 12 months					
Always	16	2	(12.5)	Referent	0.022 ^c
Sometimes	172	53	(30.8)	3.12 (0.68–14.20)	
No	289	115	(39.8)	4.63 (1.03–20.74)	
Circumcised					
No	449	162	(36.1)	Referent	0.612
Yes	39	17	(43.6)	1.37 (0.71–2.65)	
Don't know	24	8	(33.3)	0.89 (0.37–2.11)	
First pregnancy					
No	392	147	(37.5)	Referent	0.375
Yes	121	40	(33.1)	0.82 (0.54–1.27)	
Expect to deliver in the hospital					
No	5	1	(20.0)	0.44 (0.05–3.97)	0.742
Yes	500	181	(36.2)	Referent	
Don't know	5	2	(40.0)	1.18 (0.19–7.10)	
Previous breastfeeding					
No	12	5	(41.7)	Referent	0.815
Yes	321	123	(38.3)	0.87 (0.27–2.80)	
Intend to breastfeed					

Characteristics	Total no. of women	Women with a CD4+ count \leq 200 cells/mm ³		OR (95% CI)	P value ^b
		No.	(%)		
No	331	117	(35.4)	Referent	0.763
Yes	147	57	(38.8)	1.16 (0.78–1.73)	
Don't know	21	8	(38.1)	1.13 (0.45–2.79)	

Abbreviations: OR, odds ratio; CI, confidence interval; STI, sexually transmitted infection.

^aNumbers do not always total 515 or 187 (the number of women with a cell count \leq 200 cells/mm³) because of missing data.

^bValues are for the χ^2 test.

^cSignificant at 5% level.

Table 3

Correlates of low CD4+ lymphocyte count among HIV-positive pregnant women in Nigeria based on multivariate analysis and the odds associated with each variable.

Parameter	Multivariate analysis adjusted ^a	P value
	OR (95% CI)	
Age, y		
15–19	Referent	
20–24	5.23 (1.10–24.97)	0.31
25–29	6.88 (1.44–32.87)	0.03
≥ 30	7.48 (1.47–38.10)	0.03
Religion		
Christian	Referent	
Muslim	1.70 (0.95–3.02)	0.03
Other	0.36 (0.07–1.74)	0.11
Occupation		
Student	Referent	
Unemployed	1.34 (0.51–3.55)	0.96
Employed	1.86 (0.71–4.85)	0.10
Number of Pregnancies		
1	Referent	
2	0.48 (0.25–0.91)	0.037
3	0.74 (0.40–1.37)	0.92
≥ 4	0.78 (0.41–1.45)	0.71
Condom Use		
Always	Referent	
No	5.16 (1.12–23.77)	0.01
Sometimes	3.38 (0.73–15.72)	0.35
Vaginal discharge in the last 12 months		
No	Referent	
Yes	1.62 (1.06–2.48)	0.03
Ulcer in the last 12 months		
No	Referent	
Yes	1.78 (1.12–2.82)	0.02

^a All variables in the table were included in the model so the odds ratio is adjusted for the other variables (age, religion, occupation, number of pregnancies, condom use, history of discharge, and history of ulcer).