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Sociodemographic and clinical correlates of immune activation in postpartum HIV-1/HSV-2 co-infected
Kenyan women

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Abstract

Sociodemographic and clinical correlates of immune activation in postpartum HIV-1/HSV-2 co-infected
Kenyan women

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Background

HIV is a leading cause of death among women of reproductive age. An activated host immune system has been shown to support the pathogenesis and transmission of HIV-1 disease, with evidence suggesting that the activation state may be associated with social and environmental factors. Identifying these determinants of immune activation could help identify at-risk women for early intervention. This study examined the associations between sociodemographic indicators of poverty, household crowding, and poor nutritional status and markers of T-lymphocyte immune activation.

Methods

This study is nested in a recently-conducted randomized, double-blinded, placebo-controlled trial (RCT) of valacyclovir suppressive therapy among pregnant women in Nairobi, Kenya infected with both HIV-1 and HSV-2. Plasma markers of immune activation were assayed using flow cytometry at 6 and 12 months postpartum. We developed a multiple linear regression model of immune activation markers to evaluate

clinical and demographic correlates. The outcome was T-cell immune activation, as demonstrated by plasma levels of CD8⁺ and CD4⁺ cells expressing both CD38⁺ and HLA-DR⁺ proteins.

Results

The study sample comprised 117 participants. No significant association was found between sociodemographic characteristics of the participants and immune activation. Significant associations were found between factors of immune activation and both CD4 count and HIV RNA level, measured at 12 months postpartum. In models adjusting for participant age and the use of postpartum contraception, for each additional 100 CD4⁺ cell per ml, the mean CD38⁺ HLA-DR⁺ percentage was lower by 10.7% (95% CI: 0.85-0.94), and for each 10% increase in plasma RNA the mean percentage of CD4⁺ cells demonstrating CD38 and HLA-DR was higher by 3.5% (95% CI: 1.02 – 1.05).

The plasma RNA viral load was significantly positively associated with the measured percentages of activated T cells. For each 10% increase in plasma RNA concentration the geometric mean percentage of CD4⁺ cells expressing both CD38 and HLA-DR increased by 3.5% (95% CI: 1.02 – 1.05), and the percentage of CD8⁺ cells expressing these proteins increased by 3.2* (94% CI: 1.02 – 1.05).

Conclusion

This study results are consistent with earlier research associating immune activation with clinical measures of HIV disease progression, yet we found no association between measures of immune activation and sociodemographic factors in this population. Further research may help determine sociodemographic correlates of immune activation, and their association with the transmission and progression of HIV-1 disease.

Specific Aim

To determine the association between sociodemographic and clinical correlates of poverty, crowding, and nutritional status and immune activation in postpartum HIV-1/HSV-2 co-infected pregnant women in Kenya.

Hypothesis: lower socioeconomic status, household overcrowding, and poor nutritional status are associated with increased immune activation among HIV-1/HSV-2 co-infected postpartum Kenyan women.

Background

Globally, an estimated 35 million people are infected with the HIV virus, with 25 million infected in sub-Saharan Africa. HIV is the leading cause of death among women of reproductive age, and women account for a disproportionate number of new infections in sub-Saharan Africa.¹ Simple measures for estimating risk of disease transmission and progression among these women could be valuable in the allocation of resources for prevention, screening, diagnosis, and treatment of HIV-1 disease.

An activated host immune system is an important factor in the pathogenesis and transmission of HIV-1 disease, with numerous studies demonstrating higher HIV-1 viral loads and more rapid disease progression in HIV-1 infected persons with increased T cell activation.²⁻⁸ Even during treatment-mediated HIV viral suppression, the degree of immune activation prior to commencement of antiretroviral therapy is an independent predictor of clinical outcome.⁹ HIV-1 disease in turn can result in dysregulated immune activation. Coinfections are also noted to increase T cell activation and are associated with HIV disease progression.¹⁰⁻¹⁴

In a randomized controlled trial of valganciclovir vs. placebo amongst HIV-infected CMV-seropositive adult Uganda-resident participants, the valganciclovir-treated participants had significantly lower CMV DNA and lower percentages of activated CD8⁺ T-cells. The reduction in T cell activation remained significant when analysis was restricted to those with undetectable plasma HIV RNA levels. There was no significant reduction in CD4⁺ T cell counts (which decline in the context of worsening HIV disease) or increase in plasma HIV RNA levels in either arm over the 8-week trial. The study authors (Hunt et al) posit

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that these results suggest that “CMV and/or other herpesvirus coinfections are a substantial cause of *in vivo* T cell activation among treated HIV and CMV-coinfected individuals.”¹⁵

A recently-conducted randomized, double-blinded, placebo-controlled trial of valacyclovir suppressive therapy in pregnant women in Kenya (Valacyclovir in Pregnancy trial, or ViP trial) infected with both HIV-1 and HSV-2 demonstrated associations between suppressive valacyclovir therapy and reduced episodes of both genital ulcers and HSV shedding. Additionally, study participants in the valacyclovir arm had reduced HIV-1 viral load in their plasma and breast milk when compared to those receiving the placebo. The study was not sufficiently powered to detect reduction in mother to child transmission (MTCT).¹⁶

Whole blood was collected from ViP trial participants at 6 and 12 months postpartum and tested for plasma markers of immune activation for a study nested within the ViP trial. Although the study was sufficiently powered to detect a difference in mean percentage of activated CD4⁺ or CD8⁺ cells between the valacyclovir and placebo arms of the trial, no such difference was found.¹⁷

The results of the HSV-2/valacyclovir study (authored by Roxby et al) and the Hunt CMV/valganciclovir study suggest coinfection-related pathways are important in modulating immune activation among HIV-infected persons. However, the presence or absence of coinfections do not explain all of the variation in immune activation found in this population.

Studies have found higher baseline states of T-cell immune activation in residents of Africa, among both the HIV-infected and -uninfected, suggesting that the activation state may be secondary to environmental, not genetic factors.¹⁸ Notably, poor nutritional status has been associated with systemic immune activation in HIV-infected persons in Africa, and other research suggests stress as a cause of immune dysregulation ^{19,20 21,22}.

Significance

In this study, we determined whether the exposure of poverty, as measured by markers of income, crowding, access to sanitation, and malnutrition was associated with immune activation in HIV-1 infected postpartum women. Understanding the socioeconomic determinants of immune activation could help identify interventions that could improve the health status of HIV-1 infected persons. Markers of immune

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activation have been used as predictors for staging and treatment of HIV-1 infected individuals; this study aimed to add to evidence suggesting that markers of poverty may predict immune activation.

Methods

Study design

This nested study was a retrospective cohort analysis, utilizing data collected in association with the ViP trial and its nested immune activation study. The exposures measured were demographic markers of poverty, overcrowding, and poor nutrition. The outcome was T-cell immune activation, measured by serum levels of CD4⁺ and CD8⁺ T-cells expressing both CD38⁺ and HLA-DR⁺ proteins.⁸

Study setting

Participants were enrolled at the Mathare North City Council Clinic in Nairobi, Kenya. The area is one of low relative wealth; median household monthly rent among study participants was the equivalent of 23 U.S. dollars. Between April 2008 and June 2009, HIV-1-infected pregnant women seeking antenatal care at, or referred to, this facility were screened for study participation. Eligibility criteria were age \geq 18 years, seropositivity for both HIV-1 and HSV-2, at 28-32 weeks gestation, having a CD4 count $>$ 250 cells/mm³, and planning on delivering in Nairobi and residing there for 12 months postpartum. Exclusion criteria included hypersensitivity to acyclovir or valacyclovir and clinical indication for highly-active antiretroviral therapy against HIV (HAART) per WHO guidelines; at the time of the study, these guidelines recommended antiretrovirals for CD4 counts $<$ 250 cells/mm³ or diagnosis of opportunistic infections.

ViP study participants provided written informed consent at 34 weeks gestation; randomization occurred at that time, and blinding was maintained throughout the study. Sociodemographic information was first collected by interview at 34 weeks gestation, with follow-up clinical data collected at 38 weeks. Study drugs were taken from 34 weeks until 1 year postpartum. In addition to the study drug, mothers received short-course prevention of mother to child transmission (PMTCT) antiretroviral (ARV) regimens peripartum in accordance with Kenyan guidelines. For those participants who took part in the postpartum study, clinical information was also collected at 6 months and 12 months postpartum.

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Whole blood specimens were collected at all study visits; CD4 counts and HIV-1 RNA levels were determined at enrollment, 6 months, and 12 months postpartum. T cell immune activation markers were measured at 6 and 12 months postpartum. T cell activation was measured in both CD4⁺ and CD8⁺ T cells; cell count and percentage of T cells expressing CD38⁺, HLA-DR⁺, and combined CD38⁺HLA-DR⁺ were measured.

Immune activation markers were measured on fresh whole blood specimens. Specimens were surface-stained with fluorochrome-conjugated antibodies. Each specimen was run with isotype controls on a 4-color flow cytometer (FACSCalibur, Becton Dickinson). The two premixed staining combinations used were: anti-CD4-FITC/anti-CD38-PE/anti-CD3-PerCP/anti-HLA-DR-APC, and anti-CD8-FITC/anti-CD38-PE/anti-CD3-PerCP/APC. Single-stain controls and unstained controls were run daily. Flow cytometry results were interpreted using FlowJo single-cell analysis software; a logical gating strategy was used to define activated cells.¹⁷

Procedures for the primary study, including this proposed analysis, were approved by ethical review committees at the University of Nairobi and the University of Washington; all participants provided written informed consent.

Data analysis

Sociodemographic variables to be analyzed included those related to level of education, marital or polygamous status, household rent, number of residents and toilets per household, and type of toilet available. Clinical correlates included CD4 count, viral load, the number of prior pregnancies, number of live births, a history of prior STD, a history of genital ulcers, and mid-upper arm circumference (MUAC; MUAC has been studied as useful anthropometric proxy for assessment of under-nutrition in both adults and children).²³ Postpartum contraceptive use was defined as participant self-report of either of eight types of contraceptive method (Depo-Provera®, oral contraceptive pill, condom, IUD, tubal ligation, natural family planning, Norplant®, or “herbal meds”), or “not using [family planning methods]”.

Markers of immune activation were considered as continuous variables. Previous research reported higher pre-therapy CD8⁺ T cell activation levels to be predictive of subsequent mortality.⁹ The markers

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analyzed were the percentage of CD8⁺ cells expressing both CD38 and HLA-DR proteins (CD38⁺ HLA-DR⁺) and the percentage of CD4⁺ cells expressing both CD38⁺ and HLA-DR⁺.^{8,17}

Each covariate was first evaluated on its own in simple linear regression, then stepwise alongside its potential confounders and effect modifiers considered for addition to the multiple regression model one at a time for changes in the measure of association. Criteria for initial consideration in the model included P-value of the crude linear regression of less than 0.10, and a priori consideration of a correlate's importance for confounding (age). The model was then systematically re-analyzed, with potential confounders that failed to significantly affect the regression coefficients or P-values of the key correlates excluded from the model. Participant age was to be retained in the model as an *a priori* determined confounder. The use of postpartum contraception was retained in the model for significant effects on the regression coefficients and coefficients of determination. Criteria for inclusion in the final model included a P-value of less than 0.05. Analysis was performed using Stata® version 13 (StataCorp LP, College Station, Texas).²⁴

Power considerations

Power calculations incorporated assumptions of the Hunt study, notably that the standard deviation of an immune activation marker, measured as a percentage of activated T-cells, was 0.2 percentage points. For a covariate with 20% prevalence, with a desired power of 80% and Type 1 error probability of 0.05 the one-way minimal detectable difference in immune activation was calculated as 3.4 percentage points.

Results

Of the 148 women initially enrolled in the postpartum study, 12 (18%) were lost to follow-up. An additional 19 (13%) presented to clinic when the laboratory was closed. 117 (79%) had at least one immune activation result, and are described in this analysis. Summary clinical and demographic characteristics of the study subjects are displayed in Table 1.

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Table 1: clinical and demographic characteristics at baseline

Characteristic at baseline	Median (interquartile range) / n (%)
Age (years) ^a	25 (23-29)
Married	89 (76%)
No. of pregnancies	2 (1-2)
Monthly rent (KES) ^b	1700 (1200-2500)
Employed ^c	47 (40%)
Education (years)	8 (7-11)
Lifetime sex partners	2 (2-4)
History of commercial sex work	5 (4%)
HSV-2 seropositive	117 (100%)
History of genital ulcer disease	18 (15%)
WHO Stage	
Stage 1	106 (91%)
Stage 2	11 (9%)
CD4 (cells/ μ l)	367 (274-479)
Log ₁₀ HIV-1 plasma RNA (copies/ml)	3.95 (3.22-4.53)
% CD4 ⁺ cells expressing CD8 ⁺ HLA-DR ⁺	1.78 (1.08-2.49)
% CD8 ⁺ cells expressing CD8 ⁺ HLA-DR ⁺	4.7 (2.74-7.64)

^aMeasured at 34 weeks gestation

^bKenyan Shillings (KES); approx USD 23 (16-33)

^cSalaried, self-employed, or casual laborer

One hundred eleven of the 117 participants (94.9%) had an immune activation lab result at 12 months postpartum; this value was used for the analysis. Immune activation data from 6 months postpartum were used for the remaining six participants. Among the participants with both lab results available there was no significant difference in the mean percentage of activated cells between the 6-month and 12-month values.

After the initial crude linear regression analyses and diagnostics, the log-transformed versions of the output variables were selected for the final model, for improved linearity and normality.

P-values of crude associations between the exposure and output variables are shown in Table 2; for most variables there was no association between immune activation and the measured factor in unadjusted simple regression.

Table 2: measures of association of individual factors with markers of immune activation in simple linear regression.

Factor	P-value for marker associated with cell type:	
	% activated CD4 ⁺ cells	% activated CD8 ⁺ cells
Rent	0.57	0.79
Live births	0.04	0.01
Gravida	0.12	0.07
Age	0.26	0.13
Number of sex partners	0.96	0.67
Age of sexual onset	0.41	0.64
Married status	0.16	0.55
Number per household	0.04	0.17
Rooms	0.36	0.46
Years of education	0.81	0.17
Employed status	0.87	0.25
Flush toilet	0.11	0.24
CD4 count	<0.001	0.01
MUAC	0.17	0.15
RNA viral load	<0.001	<0.001
Contraception	0.01	0.05
Genital ulcer disease history	0.70	0.23

The resultant multivariable regression models are shown in Table 3.

Table 3: correlates of immune activation

1. Model of log-transformed percentage of CD4 ⁺ cells expressing both CD38 and HLA-DR			
Correlate	Coefficient	95% confidence interval	P-value
CD4 count (100 cells/ μ l)	-0.113	-0.16 – -0.07	<0.001
Age (years)	-0.027	-0.05 – 0.00	0.032
Postpartum contraceptive use	-0.277	-0.54 – -0.01	0.042
2. Model of log-transformed percentage of CD8 ⁺ cells expressing both CD38 and HLA-DR			
Correlate	Coefficient	95% confidence interval	P-value
CD4 count (100 cells/ μ l)	-0.064	-0.12 – -0.01	0.02
Age (years)	-0.028	-0.06 – 0.00	0.05
Postpartum contraceptive use	-0.271	-0.57 – 0.03	0.08
3. Model of log-transformed percentage of CD4 ⁺ cells expressing both CD38 and HLA-DR			
Correlate	Coefficient	95% confidence interval	P-value
log ₁₀ RNA (log ₁₀ cells/ml)	0.362	0.2 – 0.52	<0.001
Age (years)	-0.011	-0.04 – 0.02	0.44
Postpartum contraceptive use	-0.296	-0.57 – -0.02	0.04
4. Model of log-transformed percentage of CD8 ⁺ cells expressing both CD38 and HLA-DR			
Correlate	Coefficient	95% confidence interval	P-value
log ₁₀ RNA (log ₁₀ cells/ml)	0.336	0.17 – 0.5	<0.001
Age (years)	-0.015	-0.04 – 0.01	0.31
Postpartum contraceptive use	-0.274	-0.56 – 0.02	0.06

Sociodemographic correlates

Exposure to more live births, more pregnancies and more people per household were significantly associated with one or more marker immune activation in a univariate model. However, these associations were no longer significant when age was added to the respective models. With the exception of incidental associations noted during the modeling, no significant associations were observed with any of the other sociodemographic correlates.

Clinical correlates

The CD4 count at 12 months postpartum was significantly associated with the percentage of both Cd4 and CD8 positive CD38⁺ HLA-DR⁺ T cells ($P < 0.05$). Adjusted for participant age and the use of postpartum contraception, for each additional 100 CD4⁺ cell per ml, the geometric mean CD38⁺ HLA-DR⁺

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percentage was lower by 10.7% (95% CI: 0.85-0.94). Adjusted for participant age and the use of postpartum contraception, for each additional 100 CD8⁺ cells per ml the geometric mean CD38⁺ HLA-DR⁺ percentage was lower by 6.2% (95% CI: 0.89-0.99).

Additionally, the plasma RNA viral load was significantly associated with the measured percentages of T-cells expressing both CD38⁺ and HLA-DR⁺. Adjusted for age and the use of contraception postpartum, for each 10% increase in plasma RNA concentration, the geometric mean percentage of CD4⁺ cells expressing CD38 and HLA-DR increased by 3.5% (95% CI: 1.02 – 1.05). Adjusted for age and postpartum contraceptive use, for each 10% increase in plasma RNA concentration, the percentage of CD8⁺ cells expressing these proteins increased by 3.2% (94% CI: 1.02 – 1.05).

Discussion

This study was undertaken to investigate socioeconomic and demographic correlates of immune activation in HIV-1/HSV-2 co-infected women. The primary correlates of interest demonstrated no associations with immune activation markers, despite sufficient-power. We did observe strong associations between immune activation and both CD4 count and plasma RNA, consistent with previous studies finding worse disease status among HIV-1 infected persons with increased T cell activation. The fact that our immune activation markers tracked consistently with CD4 and viral load, as has been noted in many prior studies, shows that the lack of association of immune activation with sociodemographic and economic correlates may be true finding.

Age was included in the final model, as it has strong associations with many other correlates. Older persons are more likely to have had HIV disease for longer, in a population with similar risk factors. Older women are also more likely to have had more sex partners, pregnancies and live births. The use of contraception was also included in the model, although this too could hypothetically be associated with age or the number of sex partners a participant has had. Alternatively, certain methods of contraception may increase or decrease immune activation. More research is needed to understand this unanticipated association.

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Strengths include a sufficiently-powered study, with data collected at multiple points during pregnancy and postpartum, include a large group of African women with excellent retention.

Limitations include that immune activation was measured 12 months after the socio-demographic data was collected at study baseline, and some women may have experienced a change in their exposure to different social and economic factors in the course of the study, leading to selection bias.

Our participants were all recruited from one settlement in Nairobi and most women faced similar social and economic challenges. Since the study was performed in a geographically and sociodemographically bounded area, the relative homogeneity of participant correlates might limit the observed effect of any one of them. It may be that wider variations in socio-economic status are required to discern the effects of poverty on immune activation. Further, geographic and geospatial considerations could also limit our ability to see difference in immune activation among these women. If immune activation does indeed vary by region, a national- or regional- based sample including participants of more varied socioeconomic status may be beneficial to a future study.

Immune activation fluctuates widely during pregnancy, with lower immune activation levels recorded in the third trimester and a return to more usual levels postpartum.^{25,26} It is still unclear how long immune activation changes persist after pregnancy and how HIV affects pregnancy-related activation changes, but these issues are unlikely to affect this research, as women were all measured at the same time point – one year after delivery of their child. However, this may affect the generalizability of this data to men and non-childbearing women.

Finally, by collecting immune activation data at 12 months postpartum, we may have risked differential loss to follow up of economically deprived women who moved away or in some other way could not participate in the study, and therefore we may have lost some women who might have contributed to this analysis

In conclusion, in a large group of African women, immune activation was common and highly associated with lower CD4 counts and higher HIV RNA levels. However, we did not detect associations between exposure to poverty or social factors and immune activation. Our study was adequately powered to reject

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our hypothesis that immune activation is associated with poverty in this group of African women, but it is not clear whether this study may be generalizable to other groups of HIV positive Africans.

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Disclosure

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of Defense.

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