Herpesviruses reactivation and associated systemic inflammation following initiating ART, Rakai-Uganda

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BACKGROUND

We have previously observed an increase in vaginal shedding of HSV-2 and CMV after 4 and 8 weeks of ART initiation respectively, in women living with HIV (WLHIV) who were participating in an efficacy study of daily acyclovir to treat HSV-2. It is not clear if this reactivation is related to baseline systemic inflammation.

We conducted a prospective observational study at 3 HIV/ART clinics in south-central Uganda to evaluate HSV-2, Cytomegalovirus (CMV), and KSHV/HHV-8 herpesviruses shedding before and after ART initiation, and associated markers of systemic inflammation in WLHIV.

METHODS

- We recruited WLHIV not on ART, aged ≥18 years
- The study included a screening visit, enrolment/ART initiation/baseline visit, and visits at 2, 4, 12, 24 and 52 weeks post-ART.
- CMV and HSV-2 shedding was quantified by PCR on vaginal secretions and KSHV shedding was performed on oral swabs.
- Soluble biomarkers of systemic inflammation (IL-6, CRP, TNFα or sCD14) were measured by ELISA or Electrochemiluminescence.
- McNemar's test evaluated changes in shedding of HSV-2, CMV and KSHV pre-ART and after ART initiation (weeks 4 and 8).
- Logistic regression models were used to evaluate associations of markers of systemic inflammation at ART initiation with viral shedding at 4 and 8 weeks of ART use.

RESULTS

- 187 out of 196 (95%) screened WLHIV were enrolled and had a mean age of 30.1 years at ART initiation.
- 25.9% of participants had a pre-ART CD4 count ≤ 200 cells/μL.
- Follow-up occurred for 91% and 87% of participants at weeks 4 and 8 post-ART initiation, respectively.
- 69.1% of participants shed CMV, 44.1% shed HSV-2, and 30.1% shed KSHV
- Shedding of CMV and HSV-2, but not KSHV was observed to increase after ART initiation at week-4 and week-8 respectively **(Fig1).**

Fig1. Herpesvirus shedding during the study time points

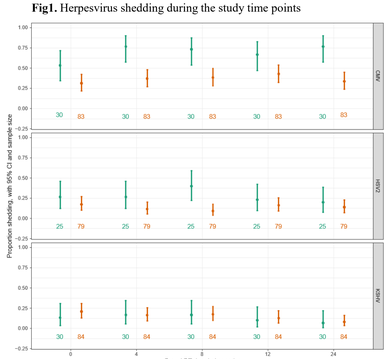
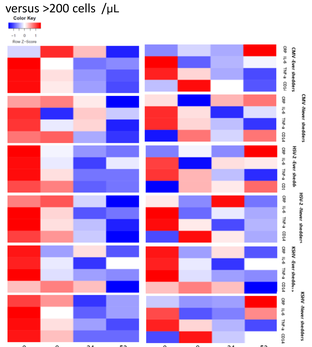


Fig2. Heatmap of median cytokine values over time by ever-shedding status and baseline CD4 count of 0-200 versus >200 cells /μL



Low CD4 counts are associated with herpesvirus reactivation after ART initiation.

ART initiation, was associated with a reduction of systemic inflammation which develops when CD4 counts have dropped below 200 cells/μL.

Levels of systemic inflammatory markers can impact the risk of mucosal shedding of CMV and HSV-2, and oral shedding of KSHV.

RESULTS

- There was consistent reduction in levels of systemic inflammation when baseline CD4 counts were ≤200 cells/μL, but not when baseline CD4 counts were >200 cells/μL **(Fig2)**.
- In participants with a pre-ART CD4 count <=200 cells/μL, shedding of HSV-2 and KSHV/HHV8 pre-ART versus at week-4 or week-8 post-ART was not significantly different; CMV shedding significantly increased from 53% pre-ART to 77% at week-4 visit (p value=0.016), and 73% at week-8 visit (p value=0.027) **(Table 1).**

Table 1: Primary Outcome Table
Proportion of women shedding herpesviruses at baseline, week 4 and 8 of ART

Herpes virus type	Baseline CD4 category	Baseline proportion (95% CI)	Week 4 proportion (95% CI)	Week 4 p-value	Week 8 proportion (95% CI)	Week 8 p-value
CMV	**0-200**	**0.53 (0.34, 0.72)**	**0.77 (0.58, 0.90)**	**0.016**	**0.73 (0.54, 0.88)**	**0.027**
	>200	0.31 (0.22, 0.42)	0.37 (0.27, 0.48)	1.000	0.38 (0.28, 0.49)	0.719
HSV2	0-200	0.27 (0.12, 0.46)	0.27 (0.12, 0.46)	1.000	0.40 (0.23, 0.59)	0.302
	>200	0.17 (0.10, 0.27)	0.12 (0.06, 0.20)	0.689	0.09 (0.04, 0.18)	0.081
KSHV	0-200	0.13 (0.04, 0.31)	0.17 (0.06, 0.35)	0.221	0.17 (0.06, 0.35)	0.683
	>200	0.21 (0.13, 0.31)	0.16 (0.09, 0.26)	0.383	0.17 (0.10, 0.27)	0.823

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Increase in baseline IL-6 levels associated with increase in HSV-2 shedding **at week-4 post ART,** if there was no shedding pre-ART, but decrease if there was shedding pre-ART . Increase in baseline CRP levels associated with increase in HSV shedding (**Table 2).**

Table 2. Association of shedding herpesviruses at week 4 of ART with the level of plasma biomarkers at baseline

Type of herpes virus at 4 weeks	Plasma Biomarker (log10 pg/mL)	Herpes virus shedding at baseline	Odds ratio	95% CI	p-value (Main effects)	p-value (Interaction effect
CMV	IL-6		1.140	(0.443, 2.952)	0.785	
	CRP		1.255	(0.706, 2.246)	0.438	
	TNF-α		3.553	(0.531, 25.765)	0.197	
	CD14		0.260	(0.016, 3.889)	0.324	
HSV2	**IL-6**	**No**	**4.428**	**(0.886, 25.102)**	**0.077**	**0.029**
		Yes	**0.233**	**(0.018, 1.683)**		
	CRP		0.711	(0.345, 1.432)	0.341	
	TNF-α		6.480	(0.586, 79.751)	0.132	
	CD14		0.268	(0.011, 8.397)	0.425	
KSHV	IL-6		1.888	(0.486, 7.357)	0.356	
	CRP		**3.259**	**(1.368, 8.419)**	**0.010**	
	TNF-α		2.286	(0.174, 30.098)	0.525	
	CD14		6.064	(0.108, 623.411)	0.417	

Increase in baseline CRP and TNFα levels associated with increase in the risk of CMV shedding **at week 8 post-ART**. Increase in baseline IL-6 and sCD14 levels associated with increase in HSV-2 shedding if there was no shedding pre-ART, but a decrease if there was shedding pre-ART **(Table 3)**.

Table 3. Association of shedding herpesviruses at week 8 of ART with the level of plasma biomarkers at baseline

Type of herpes virus at 8 weeks	Plasma Biomarker (log10 pg/mL) at baseline	Herpes virus shedding at baseline	Odds ratio	95% CI	p-value (Main effects)	p-value (Interaction effect
CMV	IL-6		1.454	(0.552, 3.926)	0.450	
	CRP		**1.826**	**(1.027, 3.358)**	**0.044**	
	TNF-α		**7.790**	**(1.117, 62.337)**	**0.044**	
	CD14		2.970	(0.194, 55.124)	0.448	
HSV2	**IL-6**	**No**	**4.372**	**(0.796, 27.187)**	**0.097**	**0.014**
		Yes	**0.173**	**(0.016, 1.163)**		
	CRP		1.096	(0.552, 2.221)	0.793	
	TNF-α		1.223	(0.121, 12.203)	0.863	
	CD14	**No**	**1.625**	**(0.031, 191.813)**	**0.829**	**0.035**
		Yes	**0.001**	**(0.000, 0.288)**		
KSHV	IL-6		0.939	(0.245, 3.463)	0.925	
	CRP		1.536	(0.697, 3.468)	0.291	
	TNF-α		1.744	(0.149, 19.703)	0.652	
	CD14		2.192	(0.063, 138.189)	0.690	

CONCLUSIONS

CD4 counts and levels of systemic inflammation before ART initiation are associated with herpes reactivation and risk of shedding after ART initiation.

