Relationship between seminal HIV-1 RNA shedding and genital schistosomiasis in HIV-positive men receiving antiretroviral therapy (ART) along the south shoreline of Lake Malawi: A prospective pilot study

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Abstract

Background: Male Genital Schistosomiasis (MGS) is hypothesized to increase seminal shedding of HIV-1 RNA. We aimed to assess the impact of MGS on genital HIV-1 RNA shedding by longitudinally measuring HIV-1 RNA levels in men established on long-term ART comparing those with and without a diagnosis of MGS.

Methods: Following optimisation of the GeneXpert® HIV-1 RNA assay for use with seminal fluid, we analysed 76 paired plasma and semen samples collected from 31 men over 12 months, comprising 15 with and 16 subjects without MGS. Study visits occurred at 0, 1, 3, 6 and 12 months. A diagnosis of schistosomiasis was based on *Schistosoma* positivity using eggs by microscopy of filtrated urine, semen, seminal sediment, POC-CCA test or by testing PCR of seminal sediment or urine at one or more time points. MGS was diagnosed by egg positivity on semen microscopy or PCR of seminal sediment. All subjects were blindly treated with a single dose of praziquantel at each study visit.

Results: Across the entire study population, HIV-1 RNA was detected in 7/76 (9.2%) seminal samples and 29/76 (38.2%) plasma samples. Among all participants, 5/31 (16.1%) showed HIV-1 RNA detection in one or more seminal samples, with 3/5 (60%) showing HIV-1 RNA detection in semen only with levels up to 400 copies/ml. The three subjects were in the MGS positive group. The two subjects showing HIV-1 RNA in seminal fluid from the MGS negative group also had concomitant HIV-1 RNA detection in plasma.

Conclusion: We found no notable difference in the pattern of genital shedding in the seminal compartment of HIV-1 infected men established on ART with and without a diagnosis for MGS following treatment with praziquantel.

Introduction

Sub-Saharan Africa (SSA) accounts for three-quarters of the global burden of HIV infection (1) and also bears a disproportionately high burden of neglected tropical diseases including schistosomiasis, a parasitic infection caused by water-borne blood flukes (2). It is estimated that of the over 200 million people infected with *Schistosoma* globally, 90% are in SSA and an estimated 6 million are HIV-positive (3, 4). A pooled analysis recently reported on the increased risk of HIV acquisition among various populations infected with *Schistosoma* (5).

In addition, HIV prevalence is often high in fishing communities of SSA where schistosomiasis is prevalent (6, 7). Women in endemic areas with female genital schistosomiasis (FGS) have been observed to have a higher HIV prevalence than others, with an increased risk of HIV acquisition by 3-fold due to the genital mucosal breach, neovascularisation and increased density of HIV receptive CD4 + cells (8–10). Genital schistosomiasis has been associated with increased seminal levels of interleukin (IL)-4, IL-6, IL-10, and tumor necrosis factor-alpha when comparing seminal egg-excreting subjects with infection-negative subjects (11). Conversely, a study in Zimbabwe examined four antiretroviral therapy (ART)-naïve HIV-positive men with MGS and demonstrated that seminal HIV-1 RNA levels declined by a median of 0.6

log_{10} copies/ml 10 weeks after anti-*Schistosoma* treatment with praziquantel (12). Whilst data is generally heterogenous across this region, one study datum showed pooled schistosomiasis prevalence of 47.4% around water reservoirs in Malawi (13).

ART suppresses HIV replication in both plasma and genital tract and reduces the risk of HIV transmission through both heterosexual and homosexual intercourse. Prospective studies of HIV discordant couples where the HIV-positive partner was established on virologically suppressive ART (defined as a plasma viral load < 200 copies/ml) showed no HIV transmission despite condomless sex (14–17). Nonetheless, detection of HIV-1 RNA in seminal fluid has been previously reported in men receiving virologically suppressive ART (18, 19). One explanation is that HIV-1 RNA suppression occurs more slowly in seminal fluid than in plasma and thus seminal shedding may be relatively common in the early phase after ART initiation (20). It has also been hypothesised that sexually transmitted and other genital infections, including male genital schistosomiasis (MGS), may promote genital shedding of HIV-1 RNA despite effective ART (21).

The aim of this study was to explore the relationship between schistosomiasis and seminal HIV-1 RNA shedding among HIV-positive men, more especially if MGS was associated with increased seminal HIV-1 RNA shedding.

Methods

Population and Sampling

The study methodology was reported previously (described in Supplementary Appendix) [19]. Study participants were HIV-1 infected heterosexual men ≥ 18 years of age who attended HIV outpatient services along Lake Malawi between November 2017 and March 2018 (Fig. 1). Participants donated paired plasma and seminal fluid prospectively over 12 months. All participants gave informed written consent and reserved the right to opt-out at any stage of the study. The disposition of the whole study population is shown in Supplementary Fig. 1. One participant with no schistosomiasis started ART on study (was excluded in analysis; unique observations have been presented in Supplementary Fig. 2). Mid-morning urine, semen and whole blood in EDTA were collected at each planned study visits. Samples were processed within 3 hours of collection.

Laboratory Procedures

Whole blood samples collected by venepuncture were centrifuged at 3000 *xg* for 5 minutes to separate the plasma for storage at − 80°C. Seminal samples were collected in a clear plastic bag following two days of abstinence from sexual activity. Following collection, seminal samples were allowed to liquefy at ambient temperature and examined under microscopy for the presence of *Schistosome* eggs, followed by centrifugation at 3000 *xg* for 5 minutes to separate supernatant seminal fluid and sediments.

The parasitological procedure was previously detailed (also in Supplementary Appendix) (22). Urine samples were tested for *Schistosoma* parasitic circulating cathodic antigen (CCA) using the point-of care parasite CCA test (POC-CCA) (Rapid Medical Diagnostics, South Africa), followed by urine filtration and microscopy for *Schistosome* eggs.

Seminal fluid was stored at − 80°C. Seminal sediment samples were re-dissolved in saline and 2–3 drops were placed on the glass slide for microscopy to detect *Schistosome* eggs. Leftover seminal sediments were preserved in ethanol and shipped to the Netherlands for *Schistosome* DNA detection by in-house real-time PCR as previously described[19]. Cryopreserved seminal fluid and plasma samples were shipped on dry ice to the UK for HIV-1 RNA testing.

Schistosomiasis diagnosis and definition of MGS

Schistosomiasis was diagnosed by *Schistosoma* positivity either by visual evidence of eggs by microscopy of filtrated urine, semen, seminal sediment; by PCR of seminal sediment, urine or stool; or by a positive POC-CCA test in urine samples. MGS was defined by positivity of *Schistosoma* either by visual evidence of eggs by microscopy of semen, seminal sediment; and / or by PCR of seminal sediment.

<insert Fig. 1>

HIV-1 RNA testing

HIV-1 RNA in collected plasma and seminal fluid was performed using the Cepheid Xpert® assay (23). The Cepheid Xpert® assay is optimised for qualitative (HIV-1 Qual assay) and quantitative (HIV-1 Viral Load assay) detection of HIV-1 in plasma (24). With 1 ml input, the HIV-1 Qual assay reports qualitative HIV-1 RNA detection with a lower limit of detection (LLOD) of 278 copies/ml; the manufacturer describes 25% detection rate at 60 copies/ml (25). With 1ml input, the HIV-1 Viral Load assay has a lower limit of quantification (LLOQ) of 40 copies/ml and a LLOD of 22 copies/ml (24). To detect HIV-1 RNA in seminal fluid, the Cepheid Xpert® assay was first validated using seminal fluid samples collected from two HIV negative donors. Validation experiments were performed with the Xpert HIV-1 Qual assay using a two-step experimental approach. Samples were spiked with the third WHO reference standard for HIV-1 RNA (NIBSC 10/152) reconstituted with molecular grade water to a concentration of 185,000 IU/ml; this is equivalent to 107,558 copies/ml using a conversion factor of 1 HIV RNA copy/mL = 1.72 IU/ml) (26). The final testing volume for testing was always 1ml. In the first set of experiments, samples were spiked to yield a final HIV-1 RNA concentration of 10000, 1000 and 100 copies/ml. This was achieved by starting with 100µl of seminal plasma, adding 93µl, 9.3µl and 0.93µl of the reference standard, and then adding water to a final 1ml volume. Due to the limited sample, each dilution point was performed as single test. In the second set of experiments, the concentration of HIV-1 RNA was kept at 291 copies/ml whereas the proportion of seminal fluid to diluent was varied. A total of 200, 300, 400, 500 and 900µl of sample were spiked with 2.7µl of reference standard, and then water was added to a final 1ml volume. This second set of experiments was performed with and without a spin step. For experiments including a spin step,

samples were spiked, diluted and then centrifuged at 500rpm for 2 minutes prior to testing. Due to the limited sample, each dilution point was performed as single test. Cycle threshold (Ct) values, i.e., the threshold where fluorescence signal exceeded the background signal, were recorded and evaluated. Following validation, the detection of HIV-1 RNA in clinical samples was performed using the Xpert HIV-1 Viral Load assay. The first set of experiments showed no loss of detection in highly diluted samples and the second set of experiments showed no loss of HIV-1 RNA detection due to inhibition at seminal fluid input volume ≥ 400µl yielding an optimal detection dilution of 2.5 folds (Supplementary Table 1). Based on sample availability, between 100µl and 400µl of seminal plasma were diluted with water to a total 1ml volume, yielding a dilution factor between 10 and 2.5 folds. The assay LLOQ and LLOD were 400 copies/ml and 220 copies/ml respectively for 10-fold dilutions and 100 copies/ml and 55 copies/ml for 2.5-fold dilutions.

Treatment

Giving the high prevalence of schistosomiasis in the region (13), participants received praziquantel at all study visits regardless of a diagnosis of MGS. After recruitment, submitting samples and receiving PZQ at baseline, participants were invited to attend planned study visits at 1 month, 3 months, 6 months and 12 months.

Statistical Analyses

Baseline characteristics by *Schistosoma* status were compared using Mann-Whitney-Wilcoxon test (continuous variables) or chi-squared/Fisher's exact test (categorical variables). Data from each group were expressed as proportions and percentages for categorical variables and median with interquartile range (IQR) for continuous variables. When describing the HIV-1 RNA results obtained with the clinical samples, results were reported as a quantified level or as an estimated level. The latter was based on the assay LLOD, which for plasma was 22 copies/ml whereas for seminal fluid ranged from 55 to 220 copies/ml according to the dilution applied. Analyses was performed using SPSS and GraphPad Prism.

Ethical Considerations

Ethical clearance was provided by the National Health Sciences Research Committee (NHSRC) of Malawi (approval number: 1805) and the Liverpool School of Tropical Medicine Research Ethics Committee (LSTM REC) (approval number: 17–018). Participants were requested for their written informed consent to be recruited and participate in the study.

Results

Study population and sampling performance

A total of 31 subjects provided at least one paired semen and blood sample over the 12 months of the study. Their baseline characteristics are summarised in Table 1. At study entry, participants had received treatment for a median of 7.5 years (IQR 1.9–13.1). Most were receiving coformulated tenofovir-DF/lamivudine/efavirenz. A total of 76 paired samples were collected, with a median of 2 paired samples per participant (range 1–5). Eleven participants donated only one set of paired samples. Overall, based on the last available paired samples, the median duration of follow-up was 10.2 months (IQR 4.0-14.2). All except 6 participants (one with schistosomiasis [A01 in Table 2] and 5 without [A17, A18, A22, A23, A26]) received PZQ treatment in the last 12 months prior to recruitment at baseline.

Table 1
Baseline demographic and HIV related characteristics of study participants

Characteristic at study entry	**Total**	***MGS* status**		***p-value***
		Positive	**Negative**	
Total number (%)	31	15	16	-
Age, median years (IQR)	46 (39, 52)	42.0 (36, 46)	51 (43, 59)	0.78
Years since HIV diagnosis, median (IQR)	7.4 (1.4, 11.7)	7.5 (11.0, 14.4)	7.8 (6.3, 11.3)	0.50
ART regimen				
TDF/3TC/EFV	25	13	12	0.53
TDF/3TC + NVP	2	1	1	-
Not known	4	1	3	-
None	1	0	1	-
ART duration, median years (IQR)	7.5 (1.9, 13.1)	5.5 (1.9, 12.0)	7.5 (5.9, 12.8)	0.44
Plasma HIV-1 RNA, median copies/ml (IQR)	816 (96, 48800)	547 (180, 15552)	26900 (53, 77900)	0.70
Plasma HIV-1 RNA detected, n (%)	16 (50)	10 (62.6)	6 (37.5)	0.28
Seminal HIV-1 RNA detected, n (%)	6 (18.8)	3 (20.0)	3 (17.6)	0.65

MGS: Male Genital Schistosomiasis; ART = Antiretroviral therapy; 3TC = Lamivudine; EFV = Efavirenz; NVP = Nevirapine; TDF = Tenofovir disoproxil fumarate. RNA = Ribonucleic acid; IQR = Interquartile range

Table 2: Overview of parasitology testing

ID	Baseline				1-month				3-months				6-months				12-months			
	UF	SEM	PCR	CCA	UF	SEM	PCR	CCA	UF	SEM	PCR	CCA	UF	SEM	PCR	CCA	UF	SEM	PCR	CCA
Schistosoma positive subjects (n=15)																				
A01	(-)	(-)	(+)	(-)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	(-)	(-)	(-)	(-)
A02	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(-)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
A03	(-)	(+)	(-)	(-)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
A04	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	NA	NA	NA	NA	(-)	(-)	(-)	(-)
A05	(+)	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)	TR	(-)	(-)	(+)	(-)	(-)	(-)	(-)	(-)
A06	(-)	(+)	(-)	(-)	(-)	(-)	NA	NA	(-)	(-)	(-)	(-)	(-)	(+)	(-)	(+)	(-)	(-)	(-)	(-)
A07	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(-)	NA	NA	NA	NA	(-)	(-)	(-)	(+)
A08	(+)	NA	NA	(+)	(+)	(-)	(+)	(-)	(+)	(+)	(+)	TR	(-)	(-)	(+)	(-)	(-)	(-)	(-)	(+)
A09	(-)	(-)	(+)	(-)	(-)	(-)	(-)	(-)	NA	NA	NA	NA	+	(-)	(-)	(-)	NA	NA	NA	NA
A10	(-)	(-)	(+)	(-)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
A11	(+)	+	(-)	(-)	NA	NA	NA	NA	NA	NA	NA	NA	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(-)
A12	(+)	(-)	NA	(-)	NA	NA	NA	NA	(+)	(-)	(+)	TR	(+)	(+)	(-)	(-)	NA	NA	NA	NA
A13	(-)	(-)	(-)	(-)	(+)	(-)	(+)	(-)	(-)	(-)	(-)	TR	(-)	(-)	(-)	(+)	(-)	(-)	(-)	(-)
A14	(+)	(+)	(+)	(-)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	(-)	(-)	(-)	(-)
A15	(-)	(-)	(+)	(-)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Schistosoma negative subjects (n=16)																				
A16	(-)	(-)	(-)	(-)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	(-)	(-)	(-)	(-)
A17	(-)	(-)	(-)	(-)	NA	NA	NA	NA	(-)	(-)	(-)	(-)	NA	NA	NA	NA	(-)	(-)	(-)	(-)
A18	(-)	(-)	(-)	(-)	NA	NA	NA	NA	(-)	(-)	(-)	(-)	NA	NA	NA	NA	NA	NA	NA	NA
A19	(-)	(-)	(-)	(-)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
A20	(-)	(-)	(-)	(-)	NA	NA	NA	NA	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
A21	(-)	(-)	(-)	(-)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
A22	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	NA	NA	NA	NA
A23	(-)	(-)	(-)	(-)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
A24	(-)	(-)	(-)	(-)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
A25	(-)	(-)	(-)	(-)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
A26	(-)	(-)	(-)	(-)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
A27	(-)	(-)	(-)	(-)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
A28	(-)	(-)	(-)	(-)	NA	NA	NA	NA	(-)	(-)	(-)	(-)	NA	NA	NA	NA	NA	NA	NA	NA
A29	(-)	(-)	(-)	(-)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
A30	(-)	(-)	(-)	(-)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
A31	(-)	(-)	(-)	(-)	NA	NA	NA	NA	(-)	(-)	(-)	(-)	NA	NA	NA	NA	(-)	(-)	(-)	TR

Bold marking indicates sample timepoint with corresponding paired HIV-1 RNA testing
Abbreviations; UF = urine filtration and microscopy; SEM = semen microscopy; CCA = circulating cathodic antigen; NA=not available; (-) = negative; (+) = positive; TR = trace

Mgs-status Definition By Parasitology Testing

Details of parasitology testing are summarised in Table 2. At baseline, there were 8 participants who tested MGS positive by semen microscopy, 4 who tested positive only by real-time PCR of seminal sediment, and 20 who tested negative by all tests. Among the 20 participants with a negative baseline test, 3 had a positive test during follow-up (1 by real-time PCR only; 1 by PCR and POC-CCA test and 1 by urine filtration, PCR and POC-CCA test) yielding a total of 15 participants who were classed as MGS positive, whereas 16 were classed as MGS negative. There were no significant differences when comparing the baseline characteristics of the two groups (Table 1).

<insert Tables 2>

HIV-1 RNA

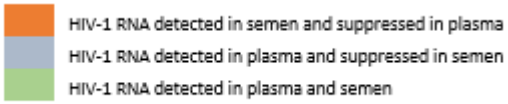
Plasma

At baseline, 5/31 (16.1%) participants on stable ART showed quantifiable plasma HIV-1 RNA (ranging from 41 to 56000 copies/ml); a further 4 showed detectable plasma HIV-1 RNA below the assay LLOQ of 40 copies/ml (Table 1). Details of HIV-1 RNA testing are shown Table 3. During follow-up, among treated participants with baseline viraemia, 1/5 achieved suppression < 40 copies/ml, 1/5 showed persistent viraemia, and 3/5 had no further plasma samples collected. Among the remaining participants who were suppressed at baseline, two showed plasma viral load rebound at month 1 (A17; 64 copies/ml) and month 3 (A20; 107,000 copies/ml) respectively, with resuppression (< 40 copies/ml) at 12 months. The participant starting ART from naïve achieved a plasma HIV-1 RNA load < 22 copies/ml by 3.7 months of treatment (Supplementary Fig. 2).

Table 3. HIV-1 RNA testing in plasma and seminal fluid by MGS status

Schistosoma positive subjects (n=15)										
Subject ID	Baseline		1-month		3-months		6-months		12-months	
	Plasma	Semen	Plasma	Semen	Plasma	Semen	Plasma	Semen	Plasma	Semen
A01	UD	UD[a]	NA	NA	NA	NA	NA	NA	D: (<40)	UD[b]
A02	UD	UD[b]	UD	UD[b]	NA	NA	NA	NA	NA	NA
A03	UD	D (220-400)	NA	NA	NA	NA	NA	NA	NA	NA
A04	UD	D (55-100)	UD	UD[b]	UD	UD[b]	NA	NA	UD	UD[b]
A05	UD	UD[b]	UD	D (55-100)	UD	123	UD	D (55-100)	UD	UD[b]
A06	UD	UD[b]	UD	UD[b]	UD	UD[b]	UD	UD[b]	UD	UD[b]
A07	D (<40)	UD[b]	UD	UD[b]	D <40	UD[b]	NA	NA	NA	NA
A08	NA	NA	UD	UD[b]	D <40	UD[b]	UD	UD[b]	UD	UD[b]
A09	D (208)	UD[b]	D (96)	UD[b]	NA	NA	D <40	UD[b]	NA	NA
A10	UD	UD[b]	NA	NA	NA	NA	NA	NA	NA	NA
A11	D (2070)	UD[b]	NA	NA	NA	NA	D (816)	UD[b]	D (277)	UD[b]
A12	UD	UD[b]	NA	NA	D (<40)	UD[b]	NA	NA	NA	NA
A13	D (<40)	UD[a]	D (<40)	UD[b]	D (<40)	UD[b]	UD	UD[b]	NA	NA
A14	UD	UD[a]	NA	NA	NA	NA	NA	NA	D (<40)	UD[b]
A15	D (56000)	UD[b]	NA	NA	NA	NA	NA	NA	NA	NA
Schistosoma negative subjects (n=17)										
A16	D (<40)	UD[b]	NA	NA	NA	NA	NA	NA	D (<40)	UD[b]
A17	UD	UD[b]	D (64)	UD[b]	NA	NA	NA	NA	D (<40)	UD[b]
A18	UD	UD[c]	NA	NA	UD	UD[c]	NA	NA	NA	NA
A29	UD	UD[c]	NA	NA	NA	NA	NA	NA	NA	NA
A20	UD	UD[a]	NA	NA	107000	528	UD	UD[b]	D (<40)	UD[b]
A21	D (26900)	D (4840)	NA	NA	NA	NA	NA	NA	NA	NA
A22	UD	UD[b]	UD	UD[b]	UD	UD[b]	UD	UD[b]	NA	NA
A23	D (<40)	UD[b]	NA	NA	NA	NA	NA	NA	NA	NA
A24	UD	UD[b]	NA	NA	NA	NA	NA	NA	NA	NA
A25	UD	UD[b]	NA	NA	NA	NA	UD	UD[b]	NA	NA
A26	UD	UD[b]	NA	NA	NA	NA	NA	NA	NA	NA
A27	UD	UD[b]	NA	NA	NA	NA	NA	NA	NA	NA
A28	UD	UD[b]	NA	NA	UD	UD[b]	NA	NA	NA	NA
A29	UD	UD[b]	NA	NA	NA	NA	NA	NA	NA	NA
A30	D (41)	UD[b]	NA	NA	NA	NA	NA	NA	NA	NA
A31	UD	UD[c]	NA	NA	UD	UD[a]	NA	NA	UD	UD[a]

UD=HIV-1 RNA undetected < 22 copies/ml; UD[a]=HIV-1 RNA undetected <110 copies/ml; UD[b]=HIV-1 RNA undetected <55 copies/ml; UD[c]=HIV-1 RNA undetected <220 copies/ml; D=HIV-1 RNA detected with viral load as copies/ml in parenthesis



<insert Table 3>

Seminal fluid

At baseline, 3/31 (9.7%) participants on stable ART showed detectable HIV-1 RNA in seminal fluid, comprising 2 MGS positive and 1 MGS negative participants. During follow-up, 2 additional participants showed HIV-1 RNA in seminal fluid, including participant A05 with persistent plasma viral load suppression (< 22 copies/ml) and participant A20 with plasma viral load rebound. Overall, 10/76 (13.2%) seminal fluid samples had detectable HIV-1 RNA. These comprised 5/10 (50%) samples with a quantified level and a median load of 1293 copies/ml (range 123-17500); 5/10 (50%) samples with detectable HIV-1 RNA at levels that were estimated < 100 copies/ml (4/10) or < 400 copies/ml (1/10), depending on the dilution factor.

Patterns of HIV-1 RNA detection by MGS status

The patterns of HIV-1 RNA detection in the 76 paired plasma and seminal samples are shown in Table 4. Overall, any HIV-1 RNA detection (with or without quantification) was concordant in 47/76 (61.8%) samples. The remaining 29/76 (38.2%) sample pairs were discordant; most had HIV-1 RNA detected in plasma only, whereas 6/76 (7.9%) had HIV-1 RNA detected in seminal fluid only. In the participant starting ART from naïve, after 1.8 months, HIV-1 RNA was qualitatively detected in plasma (estimated level 22–39 copies/ml) and still quantifiable in semen (1293 copies/ml); after 3.7 months, HIV-1 RNA was not detected in plasma (estimated level < 22 copies/ml) but was still detected in seminal fluid (estimated level 55–100 copies/ml); after 6.7 months HIV-1 RNA became also undetectable in seminal fluid (estimated level < 55 copies/ml); this subjects tested negative for MGS across all tested timepoints (Supplementary table 2).

Table 4
Patterns of HIV-1 RNA detection and quantification in paired plasma and seminal fluid

	Seminal fluid		
Plasma	Targeted not detected[a] (n)	Target Detected (n)	Quantified (n)
Target not detected[b]	43	5	1
Target detected	15	0	1
Quantified	8	0	3

[a]The lower limit of detection (LLOD) was 55 copies/ml or 220 copies/ml depending on available sample volume and therefore dilution factor required to make up 1mL input; [b]The LLOD was always 22 copies/ml

When comparing HIV-1 RNA results by MGS status, there were 16/42 (38.1%) plasma samples with quantifiable (n=6/42, 38.1%) or detectable (n=10/42, 23.8%) HIV-1 RNA among MGS positive participants, and 11/34 (32.4%) plasma samples with quantifiable (n=5/34, 14.7%) or detectable (n=6/34, 17.6%) HIV-1 RNA among MGS negative participants (Table 2). Corresponding proportions for seminal samples were 5/42 (11.9%) with quantifiable (1/42, 2.4%) or detectable (4/42, 9.5%) HIV-1 RNA among MGS positive participants, and 4/34 (11.8%) with quantifiable HIV-1 RNA among MGS negative participants. In the MGS positive group, there were 21/42 (50.0%) samples with discordant plasma / seminal fluid HIV-1 RNA results. These included 16/42 (38.1%) samples with HIV-1 RNA in plasma alone (median viral load 547 copies/ml, range 96-56000). A further 5 samples from 3 subjects had HIV-1 RNA in seminal fluid alone. In these 5 samples, HIV-1 RNA levels were 55-100 copies/ml in 3 samples, 220-400 copies/ml in one, and 123 copies/ml in one (Table 5). In the MGS negative group, there were 8/34 (23.5%) discordant samples, 7/34 (20.6%) had HIV-1 RNA in plasma alone with 1/34 (2.9%) sample from the participant starting ART naïve, having detection in semen alone. All other samples from 28 participants showing a concordant profile is shown in supplementary table 3 and 4.

<insert Table 5>

Table 5. Characteristics of the three participants with seminal HIV-1 RNA detection while plasma HIV-1 RNA was fully suppressed (<22 copies/ml)

ID	Age (years)	ART regimen	ART Duration (years)	Timepoint	Follow-up time (months)	Schisto status	HIV-1 RNA			
							Plasma	Copies/ml	Seminal fluid	Copies/ml[a]
A03	49	TDF+3TC+EFV	8.0	T0	0	+SEM	UD	<22	Detected	220-400
A04	47	TDF+3TC+EFV	11.9	T0	0	+SEM	UD	<22	Detected	55-100
			12.1	T1	2.0	-ve	UD	<22	UD	<55
			12.3	T2	3.7	-ve	UD	<22	UD	<55
			13.1	T3	13.0	-ve	UD	<22	UD	<55
A05	43	TDF+3TC+EFV	11.9	T0	0	+UF; + PCR (Ct=36.1)	UD	<22	UD	<55
			12.1	T1	2.0	Negative	UD	<22	Detected	55-100
			12.3	T2	3.7	+ve PCR: (Ct=22.5)	UD	<22	Quantified	123
			12.5	T3	6.7	+ve PCR: (Ct=23.4)	UD	<22	Detected	55-100
			13.1	T4	13.0	-ve	UD	<22	UD	<55

[a]Lower limit of quantification and lower limit of detection varied based on the dilution factor; where the HIV-1 RNA was qualitatively detected the estimated levels are

indicated. [b]Patient was ART naïve at study entry.

Abbreviations: ART=antiretroviral therapy; TDF=tenofovir disoproxil fumarate; 3TC=lamivudine; EFV=efavirenz;

UD=undetected.

Discussion

Evidence on the impact of MGS on the kinetics of HIV shedding in SSA is limited despite the significant epidemiological overlap between HIV and schistosomiasis (27). We evaluated the rates and kinetics of seminal HIV-1 RNA shedding in HIV-1 positive heterosexual men established on first-line NNRTI-based ART with and without MGS in Malawi over 12 months of follow-up and found no direct association between MGS infection and increased levels of HIV seminal shedding.

HIV co-infection with *Schistosoma* sp. infections is thought to be homologous with sexually transmitted infections (STIs) such as HSV-2; this is due to genital lesions and increased local immune responses driven by egg-induced inflammation (3) which is expected to increase the risk of HIV acquisition and propagation by increasing viral replication. Whilst data reporting the impact of *Schistosoma* sp. on genital shedding in semen is limited, one previous study evaluating the impact of urogenital schistosomiasis on HIV shedding in treatment experienced subjects showed arguably no marked decrease in seminal HIV-1 RNA levels during a follow-up period of 10 weeks (12). Of further interest are study evidence also reporting the lack of association between schistosomiasis and increased genital shedding in women (28) or association with virological/immunological failure (29).

Virologically effective ART reduces the risk of transmitting HIV sexually via both heterosexual and homosexual route (14, 15, 30) although one question remains as to the relevance of a delayed suppression in seminal fluid relative to plasma in early ART. In our study and reflective of the effectiveness of ART in suppressing HIV replication, one participant without schistosomiasis was followed prospectively after the start of therapy; the others entered the study with a median ART duration of 6.5 years (IQR: 1.9–13.0). In this participant, seminal HIV-1 RNA suppression lagged behind suppression of plasma virus and was not yet complete after 3.7 months of ART. In the study population on long-term ART, 5/31 (16.1%) participants had at least one episode of seminal HIV-1 RNA detection over 12.0 months of follow-up. Two of these subjects were negative for *Schistosoma* and had concomitant HIV-1 RNA detection in plasma however, paired samples were only available at one timepoint on study, leaving a subset of 3/31 participants (9.7%) positive for *Schistosoma* with discordant shedding. It is important to highlight that the levels of HIV-1 RNA shedding in these three participants were low and never exceeded 400 copies/ml despite all three subjects testing positive for *Schistosoma* at time of testing.

The slower decay of seminal HIV-1 RNA relative to plasma after starting ART has been previously reported (31, 32). A previous study from Kenya and Uganda evaluated the kinetics of HIV-1 RNA shedding in participants starting first-line ART (20). HIV-1 RNA was detected in 50/274 (18%) seminal fluid samples, including 37/155 (24%) collected 0–3 months after ART initiation,10% (5 of 49) collected after 4–6 months, and 11% (8 of 70) collected after more than six months. Overall rates of seminal HIV-1 RNA detection while plasma was suppressed was 16/195 (8%). Whilst the relatively high rates of HIV-1 RNA detection can be interpreted as being reflective of the early stages of treatment in these studies, rates are not dissimilar from those we observed in people on long-term ART. One other important point is related to HIV-1 RNA levels in seminal plasma. The concentration of HIV-1 RNA in the genital tract has previously been shown to correlate with the risk of HIV-1 transmission. In one study, heterosexual transmission increased by nearly 2-fold for each 1 $\log_{10}$ copies/ml increase in seminal HIV-1 RNA levels in untreated participants infected with HIV-1 and HSV-2 (33). In the study from Kenya and Uganda, majority of men shedding virus had HIV-1 RNA levels < 1000 copies; median levels during detection over the entire follow-up period was 2.96 $\log_{10}$ copies/ml (IQR, 2.51–3.50). Among the participants in this study established on long-term ART, HIV-1 RNA levels during shedding did not exceed 400 copies/ml despite positively testing for *Schistosoma.*

In two of the three subjects with longitudinal data who were both positive for *Schistosoma*, the patterns of shedding appeared to be intermittent in one and continuous in another participant, which is consistent with previous data (20), but the mechanism and significance of intermittent versus continuous shedding remains to be clarified, and may include a role of STIs in the absence of any observable effect of *Schistosoma* in this population. A previous study from Malawi evaluated the semen of HIV-positive men concomitantly diagnosed with urethritis and reported a significant decrease in seminal HIV-1 RNA shedding after antibiotic treatment (34). It is also unclear whether the shed virus is viable and infectious (19) as reported levels are typically low and whilst the precise biological mechanism to determine infectiousness remains undefined, pathogenic property of shed virus may be related to its $\log_{10}$ quantity in seminal plasma. Conversely, it is should be noted that cell-associated virus can also be potentially infectious (35).

In addition to the small number of study participants, one important limitation of this study is the lack of data on concomitant, clinically unrecognised STIs, although intermittent seminal HIV-1 RNA shedding has been reported in the absence of STIs (20). There is a close association between conditions that may cause genital mucosal inflammation and risk of HIV-transmission and one possible mechanism is through increased viral shedding. This is an important consideration as STIs are on the rise globally (36). One of such highly prevalent STIs is infection with HSV-2, which causes genital herpes. In a recent meta-analysis, it was estimated that the population attribution fraction of HSV-2 seropositivity for HIV transmission was 32% in Africa (37). Another limitation was the absence of structured adherence measures such as pill count, visual analogue scale or detection of drug levels in semen as a biological measure of treatment adherence and drug penetration into the genital compartment. It has previously

been illustrated that adherence is an independent predictor of virological failure during long-term ART (38).

One further limitation was that we only had limited seminal sample volumes and this constrained the ability to test all samples at the optimised input. In addition, there was no sample available for drug resistance analysis although drug resistance has not been reported even during continuous shedding (39). There are several points concerning the assay optimisation that should be emphasised. There was no previous literature on the use of the Cepheid Gene Xpert platform to detect and quantify HIV-1 RNA in seminal fluid. Previous studies used the Roche COBAS Ampliprep/Taqman COBAS HIV-1 test (32), Versant HIV-1 RNA 3.0 (40) assay and the Abbot m2000 system (41). In one study using the Roche COBAS Ampliprep/Taqman COBAS HIV-1 test, seminal fluid samples were diluted 2 to 3 folds to avoid inhibition (42). In studies using the Versant HIV-1 RNA 3.0 assay (43) and the Abbot m2000 system (41), semen specimens were diluted approximately six-folds. Therefore, our ability to set the dilution to 2.5-fold represents an acceptable approach in terms of sensitivity.

Conclusion

In conclusion, this study evaluated the rate and kinetics of seminal HIV-1 RNA shedding in HIV-1 positive adults established on long-term ART with and without MGS in Mangochi, Malawi and found that 9.6% of participants who were all from the MGS positive group had at least one discordant plasma undetectable/seminal fluid detectable result. Seminal HIV-1 RNA was detected at low levels with no reflection of the impact of MGS and doubtful significance in terms of risk of transmission, particularly with sporadic intermittent shedding. Further studies are required to i) evaluate the definitive role of MGS and that of STIs in enhancing HIV-1 RNA shedding in this population ii) investigate the infectiousness and drug resistance profile of seminal HIV-1 RNA and iii) determine how the introduction of dolutegravir - based ART across Africa may impact on the findings.

Declarations

Conflict of Interest

The authors have nothing to declare.

Ethics approval, consent to participate and publish the study findings

Ethics approval to conduct this study was provided by the National Health Sciences Research Committee (NHSRC) of Malawi (approval number: 1805) and the Liverpool School of Tropical Medicine Research Ethics Committee (LSTM REC) (approval number: 17-018). Eligible participants were briefed thoroughly on the study and thereafter requested to complete the informed consent in order to be recruited and participate in the study as well as publish the results.

Data Availability Statement

All data generated or analysed during this study are included in this manuscript and its supplementary information files. Any additional information can be reasonably requested from the corresponding author.

Authors' Contributions

S.A.K., A.A., J.J.K., E.J.L., A.M.G. and J.R.S. conceptualised the study; S.A.K., M.H.A., P.M., E.J.L. and J.R.S. conducted the field data collection; S.A.K. and A.A. performed the laboratory work; S.A.K, A.A, A.M.G and J. R. S. performed data analysis. S. A. K. and A. A. wrote the main manuscript text, all authors reviewed and approved the manuscript.

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Figures

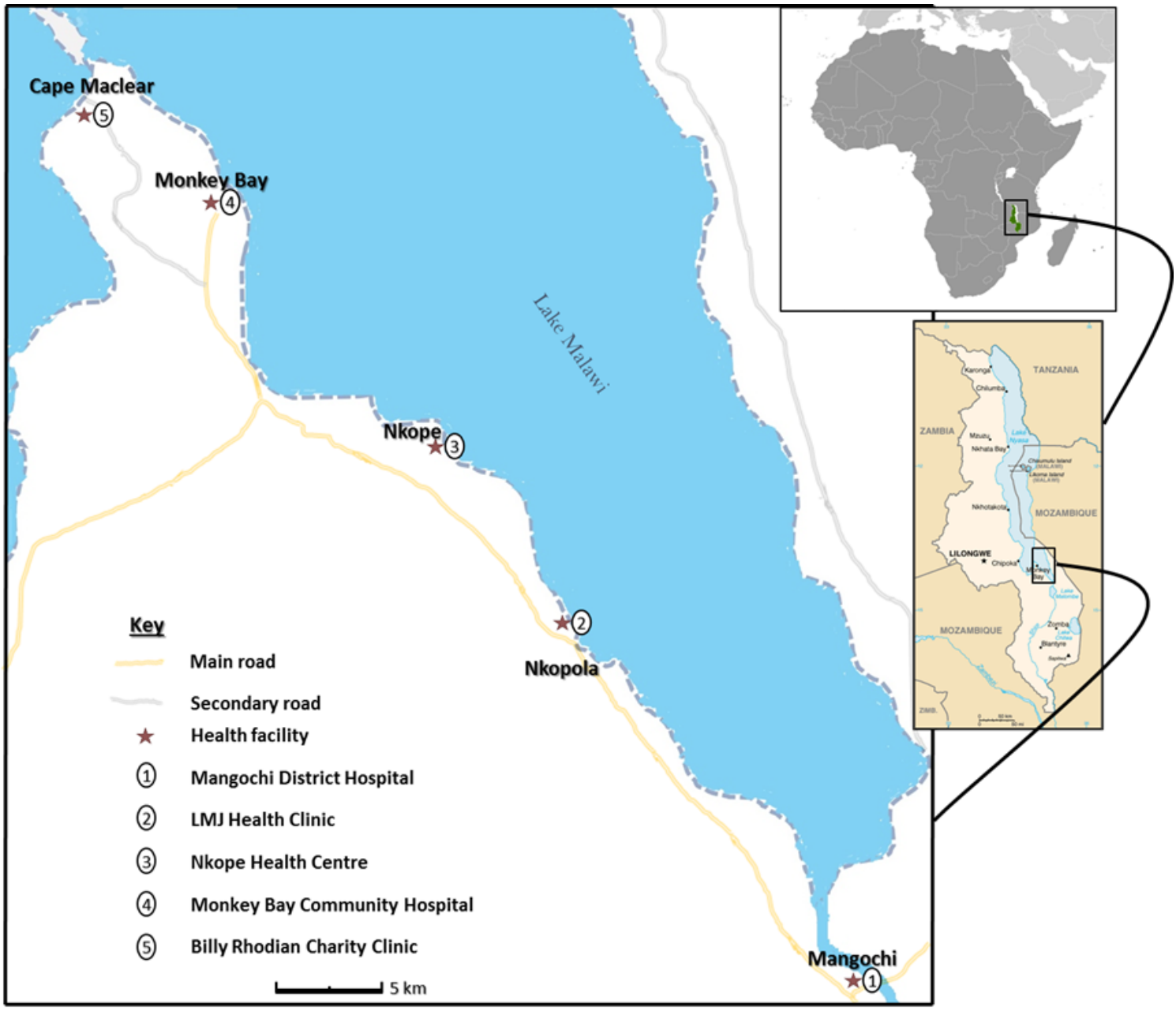

Figure 1

Schematic map of study area showing health facilities along the shores of Lake Malawi.

Supplementary Files

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