Pregnancy is associated with elevation of liver enzymes in HIV-positive women on antiretroviral therapy

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Objective: The objective of this study is to assess whether pregnancy is associated with an increased risk of liver enzyme elevation (LEE) and severe LEE in HIV-positive women on antiretroviral therapy (ART).

Design: Two observational studies: the UK Collaborative HIV Cohort (UK CHIC) study and the UK and Ireland National Study of HIV in Pregnancy and Childhood (NSHPC).

Methods: Combined data from UK CHIC and NSHPC were used to identify factors associated with LEE (grade 1–4) and severe LEE (grade 3–4). Women starting ART in 2000–2012 were included irrespective of pregnancy status. Cox proportional hazards were used to assess fixed and time-dependent covariates including pregnancy status, $CD4^+$ cell count, drug regimen and hepatitis B virus/hepatitis C virus (HBV/HCV) coinfection.

Results: One-quarter (25.7%, 982/3815) of women were pregnant during follow-up, 14.2% ($n = 541$) when starting ART. The rate of LEE was 14.5/100 person-years in and 6.0/100 person-years outside of pregnancy. The rate of severe LEE was 3.9/100 person-years in and 0.6/100 person-years outside of pregnancy. The risk of LEE and severe LEE was increased during pregnancy [LEE: adjusted hazard ratio (aHR) 1.66 (1.31–2.09); severe LEE: aHR 3.57 (2.30–5.54)], including in secondary analyses excluding 541 women pregnant when starting ART. Other factors associated with LEE and severe LEE included lower $CD4^+$ cell count (<250 cells/μl), HBV/HCV coinfection and calendar year.

Conclusion: Although few women developed severe LEE, this study provides further evidence that pregnancy is associated with an increased risk of LEE and severe LEE, reinforcing the need for regular monitoring of liver biomarkers during pregnancy.

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Introduction

Antiretroviral therapy (ART) reduces morbidity and improves the life expectancy of people living with HIV. In pregnancy, it dramatically reduces the risk of vertical transmission of the virus [1,2]. However, all ART drugs can cause hepatotoxicity of varying severity [3–6].

Alanine aminotransferase (ALT) is a biomarker of hepatic function; elevated levels indicate hepatotoxicity, or other liver dysfunction. The extent to which pregnancy affects the risk of developing ART-induced hepatotoxicity is unclear; although high rates of LEE have been observed among HIV-positive pregnant women using nevirapine (NVP) [7,8], lower rates of LEE, comparable to those seen in nonpregnant populations, have also been reported [9–11]. Cross-sectional studies comparing the rate of LEE in pregnant and nonpregnant women have also generated conflicting results [12–18]. In most of these studies, women started ART during pregnancy and follow-up ended at delivery.

We assess whether pregnancy is associated with an increased risk of LEE and severe LEE using data from women in the UK Collaborative HIV Cohort (UK CHIC) Study and the UK and Ireland National Study of HIV in Pregnancy and Childhood (NSHPC).

Materials and methods

Data collection

The UK CHIC Study is an ongoing observational study collating data from (currently 16) UK-based HIV clinics. Pseudonymized data from adults' clinical records are collected annually, including ethnicity, age, ART use, all $CD4^+$ cell counts, viral loads and liver function test (LFT) results [19]. Hepatitis B virus (HBV)/hepatitis C virus (HCV) coinfection status was determined from clinic notes or on the basis of a positive test result for HCV antibody or HBV surface antigen. The NSHPC is a comprehensive observational surveillance study of HIV-positive women accessing antenatal care in all maternity units in the UK and Ireland. Data collected include ethnicity, age and expected date of delivery, details of ART use, $CD4^+$ cell counts and viral loads in pregnancy [1]. Both studies had ethics approval. Informed consent was not required. Record linkage between these two studies (based on an algorithm using basic demographic and clinical data) has been ongoing since 2010 [20].

Inclusion criteria

The analyses included women not on ART on 31 December 1999 who started ART during 2000–2012 whilst aged 16–49 years, who had more than one ALT measurement while on ART and $CD4^+$ cell count and viral load data available during follow-up. The date of ART initiation was the baseline date and only the first initiation of ART over the study period was considered for each woman. Women with ALT more than five times the upper limit of normal (ULN) at baseline, indicating liver dysfunction, were excluded ($n = 10$). Women were included irrespective of pregnancy status/outcome or ART experience prior to 2000.

Outcomes

ALT levels were graded according to the Division of AIDS toxicity guidelines [21]. LEE (grade 1–4) was defined as at least $1.25 \times$ ULN (assumed to be 40 IU/l) among women with no evidence of LEE at baseline or at least $1.25 \times$ baseline ALT among women with ALT >ULN at baseline. Severe LEE (grade 3–4) was similarly defined using more than five-fold changes. Regimen changes (any addition or discontinuation of at least one drug in the regimen) within 3 months of incident LEE were examined.

It was assumed that women with no baseline ALT data ($n = 1856$) had ALT $\leq$ ULN. A sensitivity analysis was undertaken excluding women with no baseline ALT. As ALT is affected by plasma volume expansion (PVE), the upper limit of the reference range used in clinical practice is 8 IU/l lower in pregnancy than at other times (32 IU/l) [22]. In sensitivity analysis, the fall in ALT during pregnancy was accounted for by adding 8 IU/l to ALT measurements taken during pregnancy.

As the risk of LEE may be higher in the first few months of ART use, an increase in the risk of LEE in pregnant women may be a consequence of women starting ART antenatally. A sensitivity analysis was therefore undertaken excluding women who were already pregnant when starting ART.

Although women could act as their own controls, contributing data when pregnant and when not pregnant, some women did not have a pregnancy during follow-up. It is unlikely that all differences between women with and without a pregnancy were accounted for in adjusted analyses. Therefore, a further sensitivity analysis was undertaken excluding women with no pregnancy during follow-up.

ALT monitoring was assessed by calculating the percentage of women with at least one ALT measurement during each calendar year and the median number of measurements among these women.

Analysis

The baseline characteristics of women with and without a pregnancy at any point during follow-up were compared using chi-squared and Kruskal–Wallis tests. Follow-up started on the date of first ART and was censored at ART discontinuation, at last clinic visit or at 31 December 2012, whichever occurred first. Kaplan–Meier analyses

were used to describe the probability of LEE/severe LEE. Cox proportional hazards models were used to calculate crude and adjusted hazard ratios for the associations between factors and incident LEE/severe LEE. Fixed characteristics at baseline considered for inclusion were the pre-ART $CD4^+$ cell count (not known $\leq$250/251–350/351–500/>500 cells/μl), pre-ART viral load ($\leq$/>100 000 copies/ml), ALT within the previous 6 months, route of exposure, ethnicity and HBV/HCV coinfection. Time-dependent covariates considered, assessed at 1-month intervals, were age, pregnancy status, cumulative use of ART, latest $CD4^+$ cell count category, latest viral load category and current drug regimen (dichotomised as used/not used for each drug). Any covariates that were associated with the outcome ($P < 0.10$) in univariate models were considered for inclusion in multivariable models; covariates with a P value 0.05 or less were retained in the final model, as were age, route of exposure, ethnicity and HBV/HCV coinfection, as these were of interest for our research question. Analyses were performed using SAS (version 9.4, SAS Institute, Cary, North Carolina, USA).

Results

The 3815 women contributed 17 753 person-years of follow-up; median duration of follow-up was 4.1 [interquartile range (IQR) 1.6–7.2] years. When starting ART, the median age was 34 years, 66.0% were of black-African ethnicity, 90.6% acquired HIV heterosexually and 8.3% had HBV/HCV coinfection (Table 1). Overall, 38.3% had been diagnosed with HIV within the past 3 months and 46.5% had a $CD4^+$ cell count of 250 cells/μl or less at ART start. At baseline, 304 women had an ALT above ULN, representing 8.0% of the total or 15.5% of the 1959 women with a baseline ALT measurement.

Around one in seven (14.2%, $n = 541$) women were already pregnant when starting ART, with around a quarter (25.7%, $n = 982$) being pregnant at some time during follow-up (742 women had one and 240 more than one pregnancy). Women with a pregnancy during follow-up differed from women with no pregnancy: they were less likely to be of white ethnicity (12.7 vs. 18.6%, $P < 0.001$), to have acquired HIV via injecting drug use (IDU) (0.9 vs. 4.0%, $P < 0.001$) and be HBV/HCV coinfected (5.7 vs. 9.2%, $P < 0.001$). Women with a pregnancy were less likely to start ART with $CD4^+$ cell count 250 cells/μl or less (49.6 vs. 65.7%) and were correspondingly more likely to start with $CD4^+$ cell count more than 500 cells/μl (12.3 vs. 6.7%, $P < 0.001$). They were also less likely to have ALT above ULN at baseline (4.2 vs. 9.3%, $P < 0.001$). Women with a pregnancy were more likely to use a NVP-containing regimen during follow-up [25.3% ($n = 248$) vs. 17.1% ($n = 484$), $P < 0.001$]. Among women who started ART whilst pregnant, 23.3% ($n = 126$) used a nonnucleoside reverse transcriptase inhibitor (NNRTI)-based regimen ($n = 117$ NVP-containing) as part of their initial regimen compared with 61.3% ($n = 2008$, $n = 615$ NVP-containing) of women who were not pregnant when starting ART.

In the first 6 months on ART, the proportion of women with at least one ALT measurement was similar in both groups (63.4 vs. 65.4%, $P = 0.27$) and the median number of ALT measurements was the same [2 (IQR 0–4), $P = 0.72$]. The median number of ALT measurements undertaken in the first 6 months on ART remained stable over time (three or four for each year). ALT monitoring, in general, did not increase over time.

Incidence of liver enzyme elevation

Overall, 1080 (28.3%) women developed LEE. After 1 year on treatment, the cumulative incidence of LEE was 15% [95% confidence interval (95% CI) 14–17], increasing to 30% (95% CI 28–31) by 5 years. The overall estimated rate of LEE was 6.3 (95% CI 5.9–6.7)/100 person-years. The rate of LEE was 14.5 (11.4–17.5)/100 person-years in pregnancy and 6.0 (5.6–6.4)/100 person-years outside pregnancy. In women with HBV/HCV coinfection, 149 (47%) developed LEE, with LEE rates being 14.4 (12.1–16.7)/100 person-years in women with HBV/HCV coinfection and 5.8 (5.4–6.1)/100 person-years in women without coinfection.

In the first 6 months on ART, the rate of LEE was 21.8 (19.7–23.8)/100 person-years. For this period, the rate was higher in women who were pregnant than in women who were not pregnant [32.2 (23.9–40.5)/100 person-years vs. 20.8 (18.7–22.8)/100 person-years, respectively]. In women who had been on ART for more than 6 months, the rate of LEE was 4.2 (3.9–4.6)/100 person-years. The rate was higher in women who were pregnant than in women who were not pregnant [7.0 (4.5–9.5)/100 vs. 4.2 (3.8–4.5)/100 person-years, respectively] (Table 2).

LEE occurred during 11.6% (63/541) of pregnancies during which ART was started. In women who developed LEE during such a pregnancy, it occurred at a median of 30 (IQR 25–33) weeks gestation and 8 (IQR 4–12) weeks after ART initiation. In pregnancies conceived on ART during which LEE occurred, it occurred at median of 16 (IQR 9–28) weeks gestation.

Incidence of severe liver enzyme elevation

Overall, 151 (4.0%) women developed severe LEE. The cumulative incidence of severe LEE at 1 and 5 years after treatment initiation was 2.2% (1.7–2.7%) and 4.3% (3.5–5.0%), respectively. The overall estimated rate of severe LEE was 0.7 (0.6–0.8)/100 person-years. The rate of severe LEE was 3.9 (2.4–5.3)/100 person-years in pregnancy and 0.6 (0.5–0.7)/100 person-years outside

Table 1. Characteristics of HIV-positive women at the start of antiretroviral therapy in 2000–2012 ($n = 3815$).

Characteristic		*n*	(%)
Age, median [IQR (years)]		34	[29–39]
Exposure group	Heterosexual sex	3456	(90.6)
	IDU	122	(3.2)
	Other/NK	237	(6.2)
Ethnicity	Black-African	2517	(66.0)
	White	651	(17.1)
	Black-Caribbean	133	(3.5)
	Other/NK	514	(13.5)
HIV-HBV/HCV coinfection		317	(8.3)
Year of starting ART	2000–2002	793	(20.8)
	2003–2005	1020	(26.7)
	2006–2008	1062	(27.8)
	2009–2014	940	(24.6)
Time since HIV diagnosis	<3 months	1460	(38.3)
	3–<12 months	651	(17.1)
	1–<5 years	928	(24.3)
	≥5 years	776	(20.3)
	Median months [IQR]	7.5	[1.5–46]
$CD4^+$ cell count (cells/μl)	≤250	1774	(46.5)
	251–350	564	(14.8)
	351–500	319	(8.4)
	>500	237	(6.2)
	NK	921	(24.1)
Viral load (copies/ml)	≤400	463	(12.1)
	400–≤10 000	605	(15.9)
	10 000–≤100 000	1074	(28.2)
	≥100 000	779	(20.4)
	NK	894	(23.4)
ALT above ULN		304	(8.0)
Previous ART use		218	(5.7)
Pregnancy status when starting ART	Pregnant	541	(14.2)
	<20 weeks gestation	208	(5.5)
	≥20 weeks gestation	333	(8.7)
Type of ART regimen	NNRTI	2134	(55.9)
	PI[a]	1176	(30.8)
	NRTI[b]	130	(3.4)
	Other	375	(9.8)

ALT, alanine aminotransferase; HBV, hepatitis B; HCV, hepatitis C; IDU, injecting drug use; IQR, interquartile range; NK, not known; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside/nucleotide reverse transcriptase inhibitor; PI, protease inhibitor; ULN, upper limit of normal.
[a]One thousand and thirty-six women were on a ritonavir-boosted PI and 140 were on a nonboosted PI.
[b]This includes 68 women on zidovudine monotherapy.

pregnancy. In women with HBV/HCV coinfection, 18 (5.7%) developed severe LEE; the rates were 1.2 (0.6–1.8) in women with HBV/HCV coinfection and 0.7 (0.6–0.8)/100 person-years in women without coinfection.

In the first 6 months on ART, the rate of severe LEE was 2.9 (2.2–3.7)/100 person-years. For this period, the rate was higher in women who were pregnant than in women who were not pregnant [9.0 (4.7–13.3)/100 vs. 2.4 (1.7–3.0)/100 person-years, respectively]. In women who had been on ART for more than 6 months, the rate of severe LEE was 0.5 (0.4–0.6)/100 person-years. The rate was higher in women who were pregnant than in women who were not pregnant [2.0 (0.7–3.2)/100 vs. 0.4 (0.3–0.5)/100 person-years, respectively] (Table 2).

Severe LEE occurred during 3.3% (18/541) of pregnancies during which ART was started. In women who developed severe LEE during such a pregnancy, it occurred at a median of 30 (IQR 27–31) weeks gestation and 9 (IQR 3–12) weeks after ART initiation. In pregnancies conceived on ART during which severe LEE occurred, it occurred at a median of 24 (IQR 11–29) weeks gestation.

Factors associated with liver enzyme elevation

Being pregnant was independently associated with an increased risk of LEE (Table 2). This remained the case in further analysis excluding women who were already pregnant at ART initiation [adjusted hazard ratio (aHR) 1.91 (1.28–2.84), $P = 0.001$]. The latest $CD4^+$ cell count, but not the $CD4^+$ cell count at ART initiation, was associated with LEE with women who attained a $CD4^+$ cell count more than 500 cells/μl having a decreased risk of LEE. Women receiving zidovudine (ZDV)-containing regimens had a lower risk of LEE than women not receiving ZDV. Although women

Table 2. Rates of liver enzyme elevation and severe liver enzyme elevation per 100 person-years, with 95% confidence intervals, according to pregnancy status and duration on antiretroviral therapy.

	All women	Pregnant	Not pregnant
LEE			
Overall	6.3 (5.9–6.7)	14.5 (11.4–17.5)	6.0 (5.6–6.4)
≤6 months on ART	21.8 (19.7–23.8)	32.2 (23.9–40.5)	20.8 (18.7–22.8)
>6 months on ART	4.2 (3.9–4.6)	7.0 (4.5–9.5)	4.2 (3.8–4.5)
Severe LEE			
Overall	0.7 (0.6–0.8)	3.9 (2.4–5.3)	0.6 (0.5–0.7)
≤6 months on ART	2.9 (2.2–3.7)	9.0 (4.7–13.3)	2.4 (1.7–3.0)
>6 months on ART	0.5 (0.4–0.6)	2.0 (0.7–3.2)	0.4 (0.3–0.5)

ART, antiretroviral therapy; LEE, liver enzyme elevation.

receiving NVP or efavirenz were at an increased risk of LEE (Table 3), this risk dropped with a longer exposure to the NNRTI drug class. Other factors independently associated with LEE were HBV/HCV coinfection and having acquired HIV via IDU. There was a small, but significant, increase in the risk of developing LEE in later calendar years.

Factors associated with severe liver enzyme elevation

Factors associated with developing severe LEE were similar to those associated with developing any LEE (Table 4). Being pregnant was associated with an increased risk; this was also the case when women who started ART whilst pregnant were excluded [aHR

Table 3. Results from unadjusted and adjusted Cox proportional hazards regression analyses to identify factors associated with the incidence of any liver enzyme elevation.

		Unadjusted		Adjusted	
		HR (95% CI)	*P*	HR (95% CI)	*P*
Pregnant		1.38 (1.11–1.73)	0.004	1.66 (1.31–2.09)	<0.001
Age (per 10-year increase)		1.00 (0.92–1.08)	0.98	1.05 (0.96–1.14)	0.31
Route of exposure	Heterosexual sex	Reference	<0.001	Reference	0.02
	IDU	2.60 (2.00–3.37)		1.55 (1.12–2.15)	
	Other/NK	1.02 (0.78–1.33)		0.93 (0.71–1.22)	
Ethnicity	Black-African	Reference	0.001	Reference	0.35
	White	1.37 (1.18–1.60)		1.17 (0.98–1.38)	
	Black-Caribbean	1.08 (0.76–1.52)		1.03 (0.73–1.46)	
	Other/NK	1.09 (0.91–1.31)		1.08 (0.90–1.30)	
Calendar year		1.06 (1.03–1.08)	<0.001	1.05 (1.03–1.08)	<0.001
HBV/HCV coinfection		2.22 (1.87–2.64)	<0.001	1.85 (1.52–2.27)	<0.001
LEE at baseline		1.56 (1.21–2.01)	<0.001	–	
Latest $CD4^+$ cell count (cells/μl)	≤250	Reference	<0.001	Reference	
	251–350	0.85 (0.70–1.04)		0.82 (0.67–0.99)	0.05
	351–500	0.88 (0.73–1.06)		0.83 (0.68–1.00)	0.05
	>500	0.77 (0.63–0.93)		0.72 (0.59–0.87)	0.001
	NK	0.62 (0.52–0.76)		0.62 (0.51–0.75)	<0.001
$CD4^+$ cell count at ART start (cells/μl)	≤250	Reference	0.004	–	
	251–350	0.74 (0.57–0.96)			
	351–500	0.73 (0.58–0.92)			
	>500	0.66 (0.53–0.82)			
	NK	0.81 (0.65–1.01)			
Latest viral load (copies/ml)	≤50	Reference		–	
	>50	0.88 (0.74–1.04)	0.13		
Viral load at ART start (copies/ml)	≤100 000	Reference		–	
	>100 000	0.88 (0.70–1.10)	0.25		
ART drug in regimen	Zidovudine	0.68 (0.59–0.79)	<0.001	0.73 (0.62–0.85)	<0.001
	Efavirenz	1.00 (0.88–1.14)	0.52	1.26 (1.07–1.48)	0.005
	Nevirapine	1.02 (0.88–1.18)	0.77	1.54 (1.27–1.87)	<0.001
	Raltegravir	1.88 (1.14–3.08)	0.01	–	
Duration on ART (per additional year)		1.03 (0.95–1.12)	0.43	–	
Duration on PI regimen		1.07 (1.03–1.11)	0.001	–	
Duration on NNRTI regimen		0.94 (0.91–0.98)	0.004	0.90 (0.86–0.95)	<0.001
Duration on NRTI regimen		1.10 (0.98–1.24)	0.11		

Adjusted by covariates in the table with aHR presented. ALT, alanine aminotransferase; HBV, hepatitis B virus; HCV, hepatitis C virus; HR, hazard ratio; IDU, injecting drug use; IQR, interquartile range; LEE, liver enzyme elevation; NK, not known; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside/nucleotide reverse transcriptase inhibitor; PI, protease inhibitor; ULN, upper limit of normal.

Table 4. Results from unadjusted and adjusted Cox proportional hazards regression analyses to identify factors associated with the incidence of severe liver enzyme elevation.

		Unadjusted		Adjusted	
		HR (95% CI)	*P*	HR (95% CI)	*P*
Pregnant		3.68 (2.40–5.64)	<0.001	3.57 (2.30–5.54)	<0.001
Age (per 10-year increase)		0.74 (0.60–0.91)	0.005	0.77 (0.61–0.98)	0.04
Route of exposure	Heterosexual sex	Reference	0.09	Reference	0.16
	IDU	1.70 (0.79–3.63)		1.16 (0.46–2.93)	
	Other/NK	0.37 (0.12–1.16)		0.33 (0.10–1.06)	
Ethnicity	Black-African	Reference	0.60	Reference	0.58
	White	1.26 (0.84–1.90)		1.31 (0.84–2.04)	
	Black-Caribbean	0.86 (0.32–2.34)		0.84 (0.31–2.28)	
	Other/NK	0.89 (0.53–1.48)		0.91 (0.54–1.53)	
Calendar year		1.04 (0.99–1.10)	0.13	1.06 (1.00–1.12)	0.05
HBV/HCV coinfection		1.64 (1.00–2.68)	0.05	1.55 (0.88–2.73)	0.13
LEE at baseline		1.19 (0.59–2.43)	0.63	–	
Latest $CD4^+$ cell count (cells/μl)	≤250	Reference	0.14	Reference	
	251–350	0.61 (0.34–1.09)		0.51 (0.29–0.91)	0.02
	351–500	1.01 (0.63–1.62)		0.77 (0.48–1.24)	0.28
	>500	0.66 (0.39–1.12)		0.30 (0.30–0.84)	0.01
	NK	0.65 (0.39–1.08)		0.57 (0.35–0.95)	0.03
$CD4^+$ cell count at ART start (cells/μl)	≤250	Reference	0.97	–	
	251–350	1.11 (0.69–1.78)			
	351–500	1.19 (0.63–2.25)			
	>500	0.91 (0.40–2.10)			
	NK	1.02 (0.69–1.51)			
Latest viral load (copies/ml)	≤50	Reference		–	
	>50	0.94 (0.60–1.47)	0.79		
Viral load at ART start (copies/ml)	≤100 000	Reference		–	
	>100 000	1.01 (0.67–1.51)	0.96		
ART drug in regimen	Zidovudine	0.84 (0.59–1.20)	0.33	–	
	Efavirenz	0.75 (0.53–1.07)	0.12	–	
	Nevirapine	0.91 (0.61–1.36)	0.63	–	
	Raltegravir	2.42 (0.78–7.60)	0.13	–	
Duration on ART (per 1-year increase)		0.92 (0.76–1.11)	0.38	–	
Duration on PI regimen		1.10 (0.98–1.24)	0.10	–	
Duration on NNRTI regimen		0.89 (0.80–1.00)	0.05	–	
Duration on NRTI regimen		0.95 (0.69–1.31)	0.75	–	

Adjusted by covariates in the table with aHR presented. ALT, alanine aminotransferase; HBV, hepatitis B virus; HCV, hepatitis C virus; HR, hazard ratio; IDU, injecting drug use; IQR, interquartile range; LEE, liver enzyme elevation; NK, not known; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside/nucleotide reverse transcriptase inhibitor; PI, protease inhibitor; ULN, upper limit of normal.

4.99 (2.55–9.80), $P < 0.001$]. Calendar year, age and $CD4^+$ cell count were all associated with the risk of severe LEE, but there were no specific antiretroviral drugs that were significantly associated with severe LEE in univariate analyses. There were no deaths related to severe LEE.

Sensitivity analyses

Pregnancy remained associated with an increased risk of LEE and severe LEE when the analysis included only women who had a pregnancy during follow-up. The main findings were unaltered when women with no baseline ALT measurement were excluded and when the fall in ALT during pregnancy (due to PVE) was accounted for.

Treatment switches and interruptions among women with liver enzyme elevation

Among women who developed LEE, 5.9% (64/1080) stopped/interrupted ART and 12.0% ($n = 130$) switched regimen at a median of 44 (15–69) and 24 (9–50) days after LEE diagnosis, respectively. The percentage who altered their regimen was 14.8% (122/826) among women with ALT 50–100 IU/l, 21.0% (34/162) among women with ALT 101–200 IU/l and 41.3% (38/92) among women with ALT more than 200 IU/l (global $P < 0.001$).

Women who developed LEE in pregnancy were more likely to stop/interrupt their regimen or switch their regimen than women who were not pregnant when they developed LEE [stop/interrupt regimen: 23.9% (21/88) vs. 4.3% (43/992), respectively, odds ratio (OR) 6.92 (3.88–12.33), $P < 0.001$; switch regimen: 21.6% (19/88) vs. 11.2% (111/992), respectively, OR 2.19 (1.27–3.78), $P = 0.005$].

Among the 21 women who developed LEE in pregnancy and then stopped/interrupted ART, 20 stopped at delivery or postpartum and one interrupted ART during pregnancy. This woman conceived on an efavirenz-containing regimen, interrupted ART in the first

trimester and started a ZDV-containing regimen in the second trimester. Among the 19 women who developed LEE in pregnancy and then switched regimen, 17 switched during pregnancy, in the first ($n = 4$), second ($n = 7$) or third trimester ($n = 6$), and two switched postpartum.

Among women who developed severe LEE, 13.3% (20/151) stopped/interrupted ART and 25.2% ($n = 38$) switched regimen within 90 days at a median of 41 (17–50) days and 23 (6–51) days, respectively, after the elevated ALT measurement. The difference in the percentage of women who altered their regimen, among women who developed severe LEE in pregnancy and women who developed severe LEE whilst not pregnant was not statistically significant [stop/interrupt regimen: 22.2% (6/27) vs. 11.3% (14/124), respectively, OR 2.25 (0.78–5.41); switch regimen: 25.9% (7/27) vs. 25.0% (31/124), respectively, OR 1.05 (0.41–2.72)].

Discussion

In HIV-positive women on ART, the overall rates of LEE and severe LEE were 6.3/100 and 0.7/100 person-years, respectively. The rate of severe LEE was lower than in a study of pregnant and nonpregnant women in Côte d'Ivoire who started NVP-containing regimens [2.2 (1.1–4.0)/100 person-years] [17]. Few other studies reported LEE rates. In our study, LEE and severe LEE developed during 11.6 and 3.3% of pregnancies during which ART had been initiated, lower than that reported among pregnant women starting NVP-containing regimens [10,12] but similar to pregnant women starting nelfinavir-containing regimens [12] or when only a small proportion of women start NVP-containing regimens [18].

The initial period on ART is a time of increased toxicity risk. This was seen in our study, wherein half of the 30% of women who developed LEE within 5 years developed it within 1 year. Therefore, it is to be expected that some of the women who were pregnant when starting ART would develop LEE. However, our results suggest that pregnancy itself confers an additional risk of 70% for LEE and 260% for severe LEE. The increase in risk was apparent both in women who had recently started ART and women who had been on treatment for more than 6 months.

Some previous cross-sectional studies also adjusting for factors associated with LEE failed to observe an association between LEE and pregnancy [14,16,18], but only assessed pregnancies during which ART was started and had a short follow-up. In our study and in a U.S. study [13], which also observed an association between pregnancy and LEE, pregnancies conceived on ART were included, and in our study, women acted as their own controls by contributing data when pregnant and not pregnant. The mechanism by which pregnancy could increase susceptibility to ART-induced hepatotoxicity is not clear and may differ by ART drug [23]. The biological mechanisms that increase susceptibility to liver dysfunction during pregnancy in other diseases, such as hepatitis E, including those unique to pregnancy, such as obstetric cholestasis, are diverse and poorly understood.

As ALT is a biomarker used to indicate hepatocellular injury, LEE does not equate to ART-induced hepatotoxicity. In pregnancy, LEE could be a result of obstetric complications. However, the rate of LEE was higher than would be anticipated due to obstetric complications; a study of non-HIV-positive pregnant women observed liver dysfunction in 3% of pregnancies [24], which is thought to be similar in HIV-positive pregnant women [25].

Biomarkers, including ALT, change over the course of a pregnancy as a result of PVE [22]. In sensitivity analysis, we took into account these changes. Although this did not alter the main findings, ignoring changes in ALT that occur during normal pregnancy could mean that LEE and severe LEE are underestimated during pregnancy. Clinicians should be mindful that a lower ALT threshold may be more appropriate during pregnancy. Previous studies that did not observe an association between pregnancy and LEE may have had a different outcome if the ALT threshold for defining LEE differed according to pregnancy status [16–18]. Further work is needed to examine normal changes in ALT during pregnancy among HIV-positive women.

HBV/HCV infection can lead to LEE and is associated with an increased risk of ART-induced hepatotoxicity [26]. In our setting, HBV/HCV coinfection increased the risk of LEE 1.9-fold and severe LEE 1.6-fold. The latter association was not statistically significant, probably due to insufficient statistical power, as few women with HBV/HCV coinfection developed severe LEE ($n = 18$).

The clinical consequences of LEE are unclear; few women developed severe LEE (2% of pregnancies and 0.7% of women) and none had liver failure. Due to the risk of viral rebound, treatment changes are not recommended wherein toxicity is mild, but there is currently no agreement on how to manage pregnant women on ART who develop LEE. Close monitoring of liver biomarkers and any symptoms of toxicity, including rashes, are important [27]. Particularly with severe LEE, further tests are required, as this could indicate obstetric complications.

To minimize hepatotoxicity risk, NVP-containing regimens are not recommended for individuals starting ART

with $CD4^+$ cell count more than 250 cells/μl [28]. In our study, wherein one-fifth of women were receiving a NVP-containing regimen, higher $CD4^+$ cell count category, as a time-dependent variable, was associated with a lower risk of LEE and severe LEE, which counters the evidence that starting ART with $CD4^+$ cell count more than 250 cells/μl increases the risk of NVP-induced hepatotoxicity [14,29], although other studies have not found such an association [8,16].

As anticipated, the proportion of women who altered their ART was higher when LEE was more severe. It is not surprising that women who experienced LEE in pregnancy were more likely to stop/interrupt treatment than women who experienced LEE outside pregnancy, as many of the pregnant women would have planned to stop ART at delivery irrespective of LEE. The woman who interrupted ART during pregnancy probably did so to avoid using efavirenz [30]. It is of concern that pregnant women who experienced LEE had two times the odds of switching regimen than women who experienced it outside pregnancy.

There was a small but statistically significant increase in risk of LEE and severe LEE with increasing calendar year. This is unlikely to be due to changes in ALT monitoring, as monitoring did not increase overall or in women starting treatment. It could be due to changes in variables not measured by the UK CHIC Study but known to affect ALT such as BMI, alcohol consumption, co-medication or use of illicit drugs [31].

There were a number of other limitations to the analysis. Data were obtained from an observational study and LFTs were not performed according to a schedule but as a part of routine monitoring and where clinically indicated. This means that women with a high risk of, or with clinical indication for, hepatopathy will have had more ALT measurements. This could increase the detection rate of LEE in those with hepatopathy and therefore lead to an overestimation of the LEE incidence. In addition, HIV-positive women are more regularly monitored in pregnancy than at other times, typically having a minimum of three LFTs (at first antenatal visit, 20 weeks and 36 weeks gestation) than once every 6 months among women stable on treatment. This could introduce bias, increasing the detection rate of LEE in pregnancy. However, among women starting treatment, the average number of ALT measurements was the same in and outside pregnancy.

This study provides further evidence that pregnancy increases the risk of LEE among HIV-positive women on ART, highlighting the importance of close monitoring of ALT in pregnancy. Further work is needed to support or refute these findings and to provide clinicians with clearer guidance on the management of LEE in pregnancy.

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Conflicts of interest

All authors have no potential conflicts.

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