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ORIGINAL RESEARCH ARTICLE

Liver Injury Following Isoniazid Preventive Therapy in HIV Patients Attending Halibet National Referral Hospital, Eritrea: A Prospective Cohort Study

Mulugeta Russom[1,2,3] · Daniel Y. B. Jeannetot[2] · Araia Berhane[4] · Henok G. Woldu[5,6] · Bruno H. Stricker[7] · Katia M. C. Verhamme[2]

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Abstract

Introduction A 6-month course of isoniazid, 300 mg daily, was programmatically introduced in Eritrea in 2014 as tuberculosis preventive therapy in people living with human immunodeficiency virus (PLHIV). The rollout of isoniazid preventive therapy (IPT) in PLHIV was successful in the first 2–3 years. After 2016, rumours based on rare but real incidents of liver injuries following use of IPT spread widely across the country and created concerns amongst healthcare professionals and consumers, that ultimately caused dramatic decline in the rollout of the intervention. Decision makers have been demanding better evidence as previously conducted local studies had inherent methodological limitations. This real-world observational study was conducted to evaluate the risk of liver injury associated with IPT among PLHIV attending Halibet national referral hospital, Asmara, Eritrea.

Methods A prospective cohort study, that consecutively enrolled PLHIV attending Halibet hospital, was conducted between 1 March and 30 October 2021. Those exposed to anti-retroviral therapy (ART) plus IPT were considered as exposed and those taking only ART were considered as unexposed. Both groups were prospectively followed up for 4–5 months with monthly liver function tests (LFTs). A Cox proportional hazard model was used to explore whether there was increased risk of drug-induced liver injury (DILI) associated with IPT. Probability of survival without DILI was also estimated using Kaplan–Meier curves.

Results A total of 552 patients, 284 exposed and 268 unexposed, completed the study, with a mean follow-up time of 3.97 (SD 0.675) months for the exposed and 4.06 (SD 0.675) months for the unexposed. Twelve patients developed drug-induced liver injury (DILI), with a median time-to-onset of 35 days (interquartile range: 26.8, 60 days). All cases were from the exposed group and all except two cases were asymptomatic. The incidence rate of DILI in the exposed group was 10.6 cases per 1000 person–months and zero for the unexposed group ($p = 0.002$).

Conclusion DILI in PLHIV taking IPT was common; therefore, liver function should be closely monitored to safely administer the product. Despite high levels of deranged liver enzymes, the majority had no symptoms of DILI, emphasising the importance of close laboratory monitoring, especially during the first 3 months of treatment.

1 Introduction

Isoniazid (INH) has been one of the preferred tuberculosis (TB) preventive therapies in people living with human immunodeficiency virus (PLHIV) despite its risks of liver injury [1, 2]. The mechanism for isoniazid (INH)-induced liver injury is yet not well understood. Several pathways such as metabolite-mediated toxicity, mitochondrial damage, oxidative stress, disruption of endobiotic homeostasis and immune-mediated toxicity have been proposed as possible causes of INH-induced liver injury [3–6]. A plausible biochemical mechanism is that the metabolism of INH, a hydrazide, produces reactive or toxic metabolites that bind and damage cellular macromolecules in the liver. Three INH metabolites namely 'acetyl hydrazine, hydrazine, and a radical metabolite resulting from the bioactivation of INH itself' have been proposed to be responsible for INH-induced liver injury [2, 7].

The World Health Organization (WHO) assessed the risk of INH-induced liver injury and concluded that it was generally safe [8]. The risk of hepatic injuries following use of isoniazid preventive therapy (IPT) in PLHIV significantly varies from study to study. In some studies, IPT-related liver

Extended author information available on the last page of the article

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Key Points

Different institutions have different guidelines on the need for routine liver function monitoring while administering isoniazid preventive therapy in people living with human immunodeficiency virus (PLHIV).

This study revealed that PLHIV on isoniazid preventive therapy (IPT) were at higher risk of liver injury compared with those unexposed to IPT.

Roll out of IPT in PLHIV necessities regular laboratory monitoring of liver function tests, especially in the first 3 months of treatment to ensure safer use of the product, which urges the need for capacitating the existing laboratory setups.

injury was relatively low (0.07–2.5% [9–14]) while others have reported higher incidences (6.4–22% [15–18]).

When IPT for PLHIV was programmatically introduced in Eritrea, in 2014, increased risks of isoniazid-associated hepatic injuries, including some fatalities, were reported [17, 19]. These studies indicated that many of the cases, while on only highly active anti-retroviral therapy (HAART), had no history of drug-induced liver injury (DILI) for several years. Due to a lack of comparators or controls, the aforementioned studies could not rule out the effect of anti-retroviral therapy (ART) on DILI.

Moreover, rumours on the rarely reported cases of IPT-related hepatic injuries spread widely in the country and created concerns among healthcare professionals and PLHIV. Consequently, some healthcare professionals showed resistance against implementation of IPT that possibly caused the enormous decline in the rollout of the intervention since 2017 [20]. This put the communicable disease control (CDC) division of the Ministry of Health of Eritrea at a crossroad. Accordingly, decision makers have been demanding stronger evidence for decision making as the previously conducted studies had methodological limitations; namely they only consisted of a case series and a prospective study with active surveillance but without a comparator group [17, 19]. This real-world prospective cohort study was conducted to evaluate the risk of IPT among PLHIV attending Halibet national referral hospital located in Asmara, Eritrea.

2 Methods

2.1 Study Design and Setting

This was a prospective cohort study that consecutively enrolled PLHIV. PLHIV taking a 6 months IPT course were considered as exposed, and those without IPT as unexposed. To avoid the challenges of loss to follow-up and other competing risks, the cohort was open/dynamic, allowing patients to be recruited at different times and change their exposure status if deemed necessary by the HIV care clinic prescribers. Moreover, patients were informed on the benefits of monthly follow-ups and only those residing in the city, Asmara, were recruited to make the monthly follow-up practical. In case patients discontinued their IPT for different reasons, were lost to follow-up, died or changed their follow-up to other HIV care clinics in the country, patients were censored and only the time-period that contributed to the study was considered. The study was conducted in Halibet national referral hospital between 1 March and 30 October 2021. Halibet national referral hospital is based in the capital city of Eritrea and serves more than 1200 PLHIV; the second highest hospital in the country in serving PLHIV.

2.2 Study Population

Halibet national referral hospital has been delivering IPT to adult populations of PLHIV since November 2014. In Eritrea, once patients complete a 6 months IPT course, they are expected to take another round after 5 years of the first dose. PLHIV who had never received IPT or who had received IPT before 5 years were consecutively enrolled to the exposed group. Those who refused to be exposed to IPT or were ineligible to retake IPT (exposed to 6 months of IPT within 5 years of the commencement of the study) were also consecutively enrolled as unexposed. PLHIV who had previous history of INH-induced liver injury were excluded from the study. The study enrolled patients who had no active liver disease or elevated liver function enzymes at baseline. Thus, patients who tested positive for hepatitis B and C, had deranged liver function enzymes and a history of regular alcohol consumption were excluded from the study.

2.3 Exposure Definition and Measurement

The exposure of interest in this study was a 6 months isoniazid 300 mg tablet daily used as tuberculosis preventive therapy. PLHIV on IPT were considered as exposed and those without isoniazid as unexposed. All patients (whether exposed or not) were on ART. To minimise neurological adverse events related to isoniazid, the exposed group were also taking vitamin B6 tablets as supportive medication. Whenever required by clinicians, the study participants were free to shift their regimens of ART and/or IPT.

2.4 Outcome Definition and Measurement

The primary outcome or endpoint of this study was development of drug-induced liver injury (DILI) in the exposed and unexposed patients. Severity and classes of clinical patterns of DILI were considered as secondary outcomes.

Diagnostic criteria of DILI and its severity and clinical pattern classification were based on a consensus paper developed by a international DILI expert working group of clinicians and scientists [21]. Accordingly, DILI was diagnosed based on one of the following thresholds:

- Serum alanine aminotransferase (ALT) value ≥ 5× the upper limit of normal (ULN), or
- Alkaline phosphatase (ALP) value ≥ 2× ULN or
- ALT value ≥ 3× ULN with simultaneous elevation of bilirubin concentration to ≥ 2× ULN.

The clinical pattern of liver injury was classified according to the liver enzyme elevations that qualified as DILI [21]. Pattern of liver injury was defined using the *R*-value; where $R = (\text{ALT/ULN})/(\text{ALP/ULN})$. Estimation of ALT and ALP was made from the same serum sample. Accordingly, DILI was classified as hepatocellular pattern if $R \geq 5$, mixed pattern if $R > 2$ and < 5, and cholestatic pattern if $R \leq 2$.

As per the above-mentioned consensus paper, severity of DILI was classified as follows:

- Mild: Elevated ALT/ALP concentration reaching criteria for DILI but bilirubin concentration < 2× ULN.
- Moderate: Elevated ALT/ALP concentration reaching criteria for DILI and bilirubin concentration ≥ 2× ULN, or symptomatic hepatitis.
- Severe: Elevated ALT/ALP concentration reaching criteria for DILI, bilirubin concentration ≥ 2× ULN, and one of the following:
  - International normalised ratio ≥ 1.5, and/or
  - Ascites and/or encephalopathy, disease duration < 26 weeks, and absence of underlying cirrhosis, and/or
  - Other organ failure considered to be due to DILI.
- Fatal or transplantation: Death or transplantation due to DILI.

2.5 Patient Recruitment, Follow-Up and Study Instrument

PLHIV taking HAART were assessed for eligibility by the HIV care clinic prescriber, a medical doctor. Those eligible, as described above, and who consented to participate were subjected to baseline laboratory investigations. Patients with normal liver function tests (LFTs) and free of hepatitis B and C were consecutively enrolled to IPT. While those willing to participate but without liver diseases or deranged liver enzymes were consecutively enrolled as the comparator group (unexposed) (Fig. 1). Eligible patients were forwarded to data collectors (two registered nurse practitioners and a pharmacist working at the HIV care clinic) for enrollment. The data collectors were responsible for enrollment and follow-up of patients under the terms of the study protocol. Patients consented to participate were consecutively recruited to the exposed and unexposed groups until the required sample size was obtained. In the routine practice, patients were followed-up every 3 months, even for those taking IPT. Thus, recruitment continued for 3 months to give all patients an equal chance of recruitment.

Liver function tests such as alanine transaminase (ALT), alkaline phosphatase (ALP) and total bilirubin were performed for all study participants, during enrollment and monthly afterwards until completion of the study. In case ALT was not available, aspartate transaminase (AST) was considered. Moreover, body weight, height and CD4 cell count were taken for all patients in both groups. Drug and medical history were also assessed prior to recruitment and during follow-up. Study participants were informed on the signs and symptoms of hepatic injury such as nausea, vomiting, abdominal pain, unexplained fatigue, jaundice and dark urine, and advised to visit the health facility or call the research team in case they experienced similar events. All follow-ups were made through hospital visits.

Both exposed and unexposed groups, once enrolled, were expected to be followed-up for a period of 6 months because IPT was prescribed for 6 months. Due to a shortage of laboratory supplies impacted by the COVID-19 pandemic, the majority of the study participants completed their follow-up in 4 months. During follow-up, liver function tests (LFTs) were taken on a monthly basis for both groups. All study participants, regardless of their exposure status, were actively and equally monitored by the treating physician and data collectors for the risk of hepatic injury until the onset of hepatic injury or death, discontinuation of IPT or completion of the study, whichever came first. Patients who developed liver damage during the study period were managed according to the national treatment protocol. In all cases who developed hepatotoxicity, possible alternative explanations such as hepatitis B and C infections, alcohol consumption and intake of other drugs were assessed and ruled out.

A structured questionnaire adopted from a similar study conducted previously [17] was used to collect data. The questionnaire had four main sections: patient demographic characteristics, drug details, baseline and follow-up test results, and reaction (liver injury) details. Data

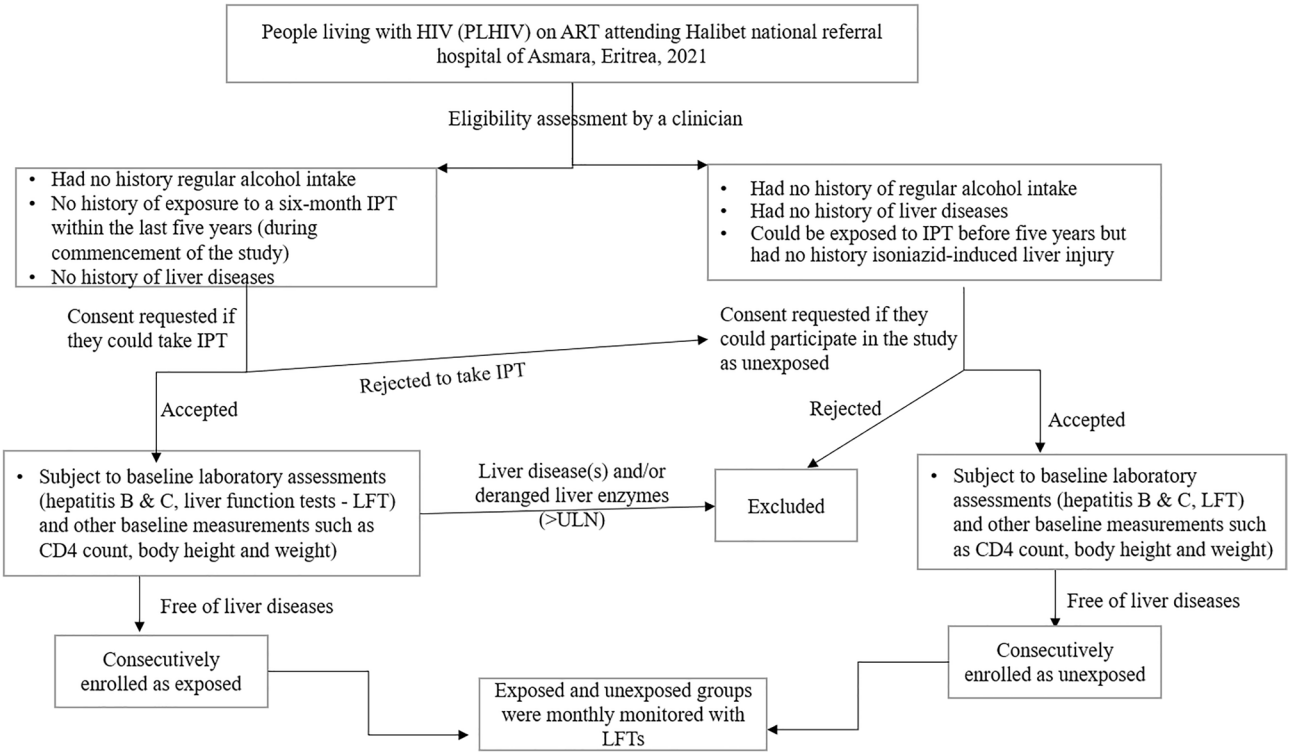

Fig. 1 Eligibility criteria for enrollment and approach of PLHIV attending Halibet national referral hospital, 1 March–30 October 2021, Asmara, Eritrea. IPT: Isoniazid preventive therapy; LFT: Liver function test; HAART: Highly active anti-retroviral drugs; ULN: Upper limit of normal

were collected by the data collectors working in the HIV care clinic and supervised by one of the authors (M.R.).

2.6 Sample Size Determination

For this cohort study, sample size was calculated to be 502 (251 from each group) with Epi Info version 7.2 [22] with the following assumptions: Margin of error: $\alpha = 0.05$; statistical power: 90%; exposed to unexposed ratio: 1 and assumed relative risk: 2.5. Besides, as there were no local studies that evaluate the incidence of hepatic injury associated with HAART medicines, a median expected cumulative incidence of hepatic injury in the unexposed population (HIV patients without IPT) was taken from the literature as 8.4% [23]. Including 10% for non-response, the sample size was finally calculated to be 552 (276 from each group).

2.7 Data Analysis

Data were entered using a program census and survey processing system (CSPro) version 7.4 and analysed using R 4.0.4 [24] and SPSS 20.0. Descriptive statistics including percentages, frequencies, number needed to harm, mean (standard deviation, SD) and median (interquartile range, IQR) were computed as appropriate. As a measure of frequency, incidence rate of DILI was computed using descriptive statistics. Chi-square tests of independence and bivariate logistic regression were used to identify factors associated with DILI. A Cox proportional hazard model was used to explore whether there was increased risk of drug-induced liver injury (DILI) associated with IPT and compare the time-to-event onset of DILI in both groups (time-to-event analysis). To compare the incidence rate of DILI in the exposed versus the unexposed, the rate ratio (RR) or the hazard ratio with 95% confidence interval (CI) was computed, as appropriate. Calculating the RR/HR to compare risk of DILI in the exposed and unexposed was not applicable or was undefined as none of the unexposed group could develop at least one case of DILI. Even though it is unknown how many cases from the exposed group would develop DILI until at least one case was observed in the unexposed, a sensitivity analysis was carried out to calculate the lowest possible RR by adding one case to the unexposed group. A two-tailed test with a 0.05 alpha level of significance was used for all statistical tests.

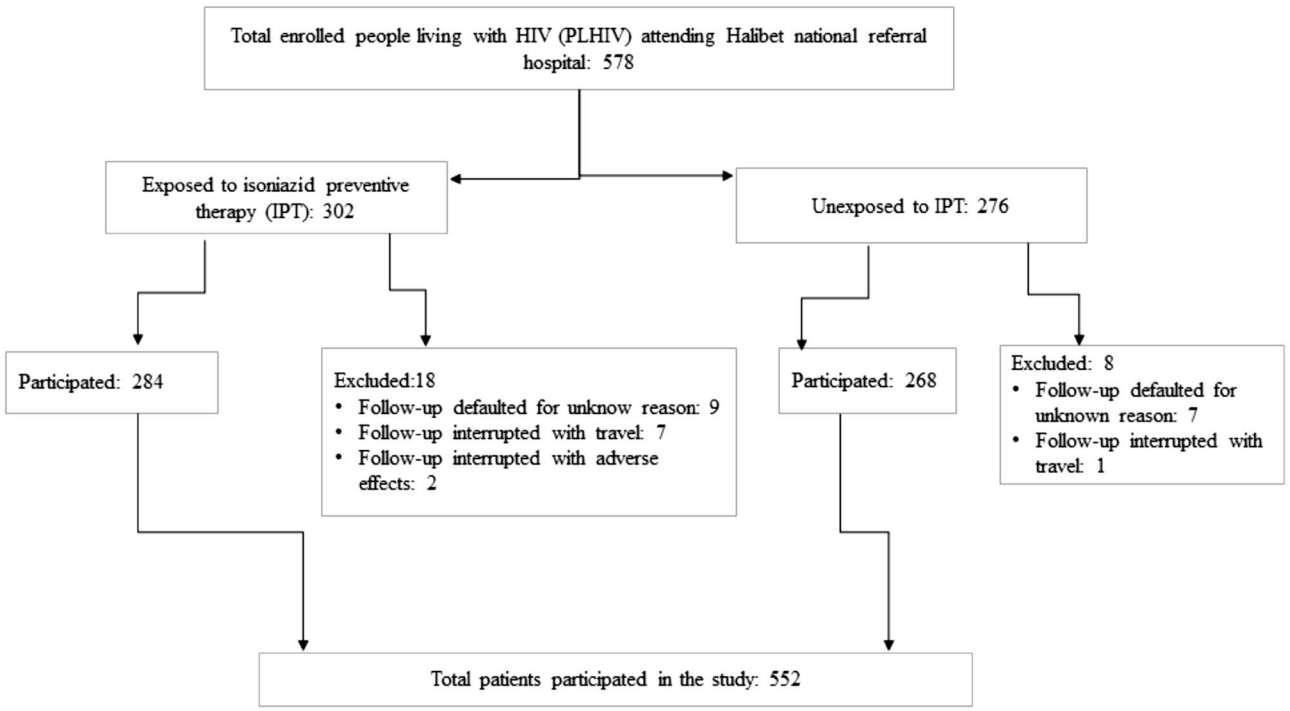

Fig. 2 Enrollment and follow-up information of PLHIV attending Halibet national Referral hospital, 1 March–30 October 2021, Asmara, Eritrea

3 Results

3.1 Background and Follow-Up Information of Study Participants

A total of 578 PLHIV on ART, 302 (51%) exposed and 276 (49%) unexposed, were enrolled into the study (Fig. 2). The exposed group had a median age of 48 years [interquartile range (IQR): 43, 53 years] and for the unexposed group it was 47 years (IQR: 41, 52 years). About two-thirds (67.2%) of the study participants were females (Table 1). The exposed and unexposed groups had no differences in baseline characteristics except in history of comorbidities ($p = 0.015$) and type of ART which was taken during the study period ($p < 0.001$). The majority of the study participants were on efavirenz-based (58.3%) and 40.2% were on a dolutegravir-based ART regimen. Of those exposed to IPT, 65.5% and 32.7% were on efavirenz-based and dolutegravir-based ART regimens, respectively, while in the unexposed group the proportion was 50.7% for efavirenz-based regimen and 48.1% for dolutegravir-based regimen.

There was no difference in follow-up time between groups ($p = 0.109$). The mean follow-up time for the exposed group was 3.97 (SD 0.68) months, and 4.06 (SD 0.68) months for the unexposed (Table 2).

3.2 Incidence Rate of Hepatic Injury Associated with IPT

The cumulative follow-up time in the exposed and unexposed groups were 1128 and 1089 months, respectively. In this time period, a total of 12 study participants, all from the exposed group, developed the outcome of interest (DILI). None of the unexposed group developed DILI. The incidence rate of DILI in the exposed group was 10.6 cases per 1000 person–months and in the unexposed group it was zero (0) cases per 1000 person–months (Table 3). The exposed group were likely to develop DILI compared with the unexposed ($p = 0.002$), even though it was not tested at the multivariate level for reasons explained in the data analysis section.

3.3 Sensitivity Analysis

Rate ratio was calculated by adding one case to the unexposed group to estimate the lowest relative risk of DILI and was found to be: (RR: 11.32; 95% CI: 1.68, 484.07).

3.4 Description of the DILI Cases

Cases of DILI occurred in ten females and two males. Their median ALT and ALP activity was 7.3 (IQR: 5.4, 13.8) and 0.85 (IQR: 0.63, 1.38), respectively. The median *R*-value of

Table 1 Sociodemographic characteristics of PLHIV, both exposed and unexposed cohorts to isoniazid preventive therapy, attending Halibet national referral hospital, Asmara, Eritrea, 2021 ($N = 552$)

Variables *(measured at baseline)*	Category	Cohort group		*p* value
		Exposed *n* (%) *n* = 284	Unexposed *n* (%) *n* = 268	
Age group (years)				
	15–59	266 (93.7)	244 (91.1)	0.246
	60 and above	18 (6.3)	24 (8.9)	
Sex				
	Male	94 (33.1)	87 (32.5)	0.874
	Female	190 (66.9)	181 (67.5)	
Body weight (kg)				
	< 50	60 (21.1)	74 (27.6)	0.076
	50 and above	224 (78.9)	194 (72.4)	
BMI				
	< 18.5	50 (17.6)	54 (20.2)	0.526
	18.5 to < 25	179 (63.0)	167 (62.3)	
	25 to < 30	40 (14.1)	38 (10.3)	
	30 and above	15 (5.3)	8 (3.0)	
Co-morbidity				
	Yes	18 (6.3)	33 (12.3)	0.015
	No	266 (93.7)	235 (87.7)	
CD4 cell count (cell/μL) (just before or at enrollment)[α]				
	< 200	15 (5.3)	28 (10.4)	0.039
	> 200	259 (91.2)	234 (87.3)	
Type of ART*				
	Nevirapine based	5 (1.8)	1 (0.4)	< 0.001
	Efavirenz based	186 (65.5)	136 (50.7)	
	Dolutegravir based	93 (32.7)	129 (48.1)	

PLHIV: People living with HIV; IPT: Isoniazid preventive therapy; SD: Standard deviation; IQR: Interquartile range; ART: Anti-retroviral therapy

*Few patients on other ART regimens are removed from this analysis as they were few in number

[α]For some patients CD4 cell count was missing

the cases was 8.8 (IQR: 6.3, 14.2). In all cases except one, the type of pattern of liver injury was hepatocellular. Severity of the DILI was mild in ten cases and in the other two it was moderate (Table 4). All but two cases were asymptomatic. The time to onset, the time between initiation of INH and first diagnosis of DILI, was 35 days (IQR: 26.8, 60 days). The association of DILI with IPT was probable in all cases. A symptomatic treatment, a vitamin B complex tablet, was provided to two patients with DILI, and none of the other cases received treatment. In all cases, DILI was reported as either recovered ($n = 9$) or recovering ($n = 3$). As part of management of DILI, only IPT was withdrawn in all the cases.

3.5 Survival Analysis

Kaplan–Meier estimation shows that the unexposed group had higher survival probability without DILI compared with the exposed (Fig. 3). At about five months follow-up, the probability of survival without DILI for the unexposed was 100% while for the exposed group, it was estimated to be around 93%.

4 Discussion

In this real-world observational cohort study, a higher risk of DILI was observed in the exposed group but its statistical significance at a multivariate level could not be explored for reasons reported in the data analysis of the methods section. Nevertheless, considering the life-threatening nature of the outcome, observing 12 cases (4.2%) only in the exposed group is clinically important and of concern. All possible causes that could be potentially hepatotoxic were assessed and no alternative explanation was found for all cases. However, we acknowledge that other unmeasured confounders may still exist, which is an inherent limitation of such studies. Comparing this finding with studies reported elsewhere is a challenge due to variability of the results. A recent meta-analysis and systematic review of individual participant data from randomised controlled trials reported a 2.5% (range: 0.83–9.68%) incidence rate of hepatic injuries following the use of IPT in PLHIV [9]. While different observational studies that assessed the risk of IPT-induced hepatic injury in PLHIV reported relatively higher but variable incidences (4.9–22% [12, 16, 17]). The difference in cumulative incidences of DILI associated with IPT in PLHIV might be related to the diagnostic criteria used, study designs employed, length of use of IPT, duration and strictness of follow-up, other concomitant drugs used, patients' clinical conditions and so on. Reporting inconsistent results of DILI due to differences in diagnostic criteria have been of concern [25], which could result in inappropriate decision making.

In this study, in almost all cases, the pattern of liver injury was hepatocellular; none of them were cholestatic. Such a pattern is in-line with INH-related hepatotoxicity. Besides, the majority of the cases were asymptomatic despite high levels of ALT and all except one developed the outcome within 2 months, which is consistent with a previously conducted local study [17]. This reflects that, in a situation where there is no close laboratory monitoring, patients might come to health facilities with severely elevated liver enzymes before they show clinical manifestations of liver injury. This could be even worse if the follow-up time is

Table 2 Follow-up outcome characteristics of PLHIV, both exposed and unexposed cohorts to IPT, attending Halibet national referral hospital, Eritrea, 2021($N = 552$)

Variables *(Measured at baseline)*	Category	Cohort group		*p* value
		Exposed *n* (%) $n = 284$	Unexposed *n* (%) $n = 268$	
Cumulative exposure to ART in years (before and during study)				
	2 and below	99 (35.2)	141 (52.8)	< 0.001
	2–5	10 (3.6)	21 (7.9)	
	> 5	172 (61.2)	105 (39.3)	
Mean follow-up time (in months)		3.97	4.06	0.109
Follow-up status				
	Follow-up completed	284	268	0.097*
	Follow-up defaulted with unknown reasons	9	7	
	Follow-up interrupted with travel	7	1	
	Follow-up interrupted with adverse event(s)	2	0	

PLHIV: People living with HIV; IPT: Isoniazid preventive therapy; SD: Standard deviation; IQR: Interquartile range; ART: Anti-retroviral therapy

*Fisher's exact test

every 2–3 months, which was a common practice in Eritrea before and during the study period. Thus, relying mainly on clinical monitoring, as specified in national, International Union against Tuberculosis and Lung Diseases and WHO guidelines on implementation of IPT [8] could make it challenging and emphasises the need for regular laboratory monitoring (LFT) especially during the first 3 months of follow-up. A recently conducted qualitative study, aimed at determining the factors that affected implementation of IPT in Eritrea, revealed that inadequate laboratory setup, especially at subnational levels, was among the root causes for limited deployment of IPT in Eritrea [20].

Findings of a systematic review performed to understand the best clinical approach used in monitoring toxicity during the use of tuberculosis preventive therapies revealed that the existing monitoring strategies are not standardised and largely dependent on availability of resources, expert opinion and clinical practices [26]. The American Thoracic Society (ATS) and the Centers of Disease Control and prevention (CDC) of the USA, as well as the national guidelines of Portugal, Ireland and Sweden recommend at least monthly LFT monitoring during follow-up for PLHIV taking TB preventive therapy [26]. This is in-line with the recommendation of market authorization holders of isoniazid 300 mg tablet that advises regular monitoring of liver function (weekly during the first month, and then monthly) at least for the first 3 months of treatment [27, 28]. The systematic review also highlighted that the decision on intervals of LFT monitoring among different institutions/countries varied, which emphasizes the need to standardize frequency of LFT monitoring based on availability of resources, risk factors and so on. The systematic review also reflected that France and Canada recommend monthly LFT monitoring, regardless of their HIV status, for people on TB preventive therapy aged more than 50 and 65 years, respectively [26]. The Médicins Sans Frontierès guidelines on tuberculosis also advises that baseline LFTs can be performed in PLHIV starting TB preventive therapy, depending on the availability of resources, while follow-up tests are required if patients develop signs of hepatic injury [29].

This study showed that in all cases, because of fear of complications, IPT was discontinued even though the hepatic injury was mild in the majority. That being said, in randomised controlled trials, withdrawal of IPT following DILI was reported to be low (2.9–4.7% [13, 15]). This could possibly be related to the nature of the controlled environment that enabled investigators to closely monitor and follow-up patients. In our case, to minimise unnecessary treatment interruptions that could lead to suboptimal treatment outcomes and antibiotic resistance, efforts should be made to improve adherence to local and/or international guidelines on when to stop a suspected offending drug. A recently published guideline highlights that DILI could be transient and asymptomatic, and thus, interruption or change of treatment is not required unless there are signs of anorexia, malaise, vomiting or clinically evident jaundice [30].

The results of this study could have the following programmatic and policy implications. Deployment of IPT in PLHIV necessities regular laboratory monitoring of LFTs, especially in the first 3 months of treatment to ensure safer use of the product. This urge ensuring the existing laboratory setup have the capacity, including those in the remote areas, to minimise IPT implementation practicality challenges. Taking into consideration that the cases

Table 3 Incidence rate of hepatic injury associated with isoniazid preventive therapy (IPT) and of associated factors in PLHIV attending Halibet national referral hospital in 2021 ($N = 552$)

Variables	Cases of hepatic injury *n* (%)	Person–time (months)	Incidence rate per 1000 person–months	Crude rate ratio (95% CI)	*p*-value
Exposure to *IPT					
Exposed	12 (4.93)	1128	10.60	*Not applicable*[¥]	–
Unexposed	0 (0.00)	1089	0.00		
Age (years)*					
≤ 45	4	802	4.99	*Ref*	0.099
> 45	8	1415	5.62	*1.33 (0.303, 5.144)*	
Sex					
Female	10	1491	6.71	*2.67 (0.58, 24.86)*	0.244
Male	2	726	2.75	*Ref*	
BMI					
< 18.5	4	418	9.57	*0.87 (0.08, 42.64)*	0.998
18.5 to < 25	5	1396	3.58	*0.32 (0.04, 15.33)*	0.314
25 to < 30	2	309	6.48	*0.59 (0.03, 34.59)*	0.538
30 and above	1	90.5	11.0	*Ref*	
Total number of drugs taken*					
≤ 2	0	1062	0.00	*Ref*	
3–4	10	1105	9.05	*10.57 (1.52, 455.05)*	0.006
> 4	2	50.1	39.9	*42.39 (2.21, 501.19)*	0.006
CD4 cell count (cell/μL)*					
< 200	0	177	0.00	*1.01 (0.02, 6.96)*	0.999
> 200	10	1974	5.07	*Ref*	
Type of ART regimen					
Nevirapine based	1	22.7	44.1	*8.14 (0.17, 72.75)*	0.135
Efavirenz based	7	1262	4.75	*0.88 (0.22, 3.64)*	0.998
Dolutegravir based	6	924	5.41	*Ref*	

PLHIV: People living with HIV; IPT: Isoniazid preventive therapy; Ref: Reference category; ART: anti-retroviral therapy; CI: Confidence interval

[¥]Crude rate ratio could not be calculated as the incidence rate in the unexposed was zero

*For two patients, CD4 cell count was not available

were asymptomatic and all recovered following withdrawal of the offending drug (IPT), someone might argue on the need for or cost-effectiveness of introduction of routine laboratory monitoring. It should, however, be well noted that the cases were closely monitored and intervention was discontinued when the liver function markers were noticeably elevated. In routine practice, patients are followed-up every 3 months which exposes them to the risk of liver injury complications, including death. Though implementation of routine laboratory monitoring might necessitate a formal cost-effectiveness assessment, the authors believe that it is applicable for the following reasons: the number people living HIV on ART in Eritrea is low (around 9000); the national HIV/AIDS control programme is well funded which could be helpful in capacitating the health system; the requested routine laboratory monitoring is short (at least 3 months); and the majority of the HIV patients (> 75%) are attending in the national and regional referral hospitals where a chemistry laboratory is available.

The study had the following limitations. Even though sample size was reasonably calculated, the fact that all of the cases were from the exposed group made the calculation of RR/HR not possible. Accordingly, a sensitivity analysis was performed by adding one case to the unexposed group to predict the lowest possible RR. Besides, considering the risks of INH-induced liver injury, participants exposed to IPT might have been closely followed, rigorously investigated, and strictly advised, compared to the unexposed, which might have affected the diagnosis rate of the outcome of interest. Efforts were made to follow the same protocol, for both groups, but the data collectors might have intuitively introduced a diagnostic suspicion bias. Due to shortage of laboratory consumables, impacted by COVID-19 restrictions, monitoring LFTs for

Table 4 Overview of the cases who developed isoniazid-induced hepatic injury in PLHIV attending Halibet national referral hospital, followed-up between 1 March and 30 October 2021 ($N = 552$)

Case no.	Age	Sex	Exposure status	ALT	ALP	Total bilirubin	INR	ALT ULN	ALP ULN	ALT activity	ALP activity	*R*-value	TTO	Symptomatic?	Pattern of liver injury	Severity
1	57	F	Exposed	583	389	1	1.078	41	153	14.22	2.54	5.59	90	No	Hepatocellular pattern	Mild
2	38	F	Exposed	262	80	0.8	N/A	41	153	6.39	0.52	12.22	60	No	Hepatocellular pattern	Mild
3	51	F	Exposed	291	121	0.7	N/A	41	153	7.10	0.79	8.97	60	No	Hepatocellular pattern	Mild
4	54	M	Exposed	480	251	1	N/A	41	153	11.71	1.64	7.14	39	No	Hepatocellular pattern	Mild
5	41	F	Exposed	206	90	0.5	N/A	41	153	5.02	0.59	8.54	34	No	Hepatocellular pattern	Mild
6	42	F	Exposed	515	132	0.6	N/A	41	153	12.56	0.86	14.56	60	No	Hepatocellular pattern	Mild
7	49	F	Exposed	1,256	226	3.5	N/A	41	153	30.63	1.48	20.74	22	Yes	Hepatocellular pattern	Moderate
8	47	F	Exposed	207	90	0.5	1.019	41	153	4.93	0.59	8.38	26	No	Hepatocellular pattern	Mild
9	59	M	Exposed	839	161	0.8	1.136	41	153	20.46	1.05	19.45	36	No	Hepatocellular pattern	Mild
10	38	F	Exposed	1,115	165	1	N/A	41	153	27.20	1.08	25.22	27	Yes	Hepatocellular pattern	Moderate
11	59	F	Exposed	277	246	0.8	N/A	41	153	6.76	1.61	4.20	33	No	Mixed pattern	Mild
12	54	F	Exposed	309	89	0.4	N/A	41	153	7.54	0.58	12.96	27	No	Hepatocellular pattern	Mild

ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; INR: International normalized ratio; ULN: Upper limit of normal; ALT activity: ALT/ULN; ALP activity: ALP/ULN; *R*-value: ALT activity/ALP activity; TTO: Time to onset (in days)

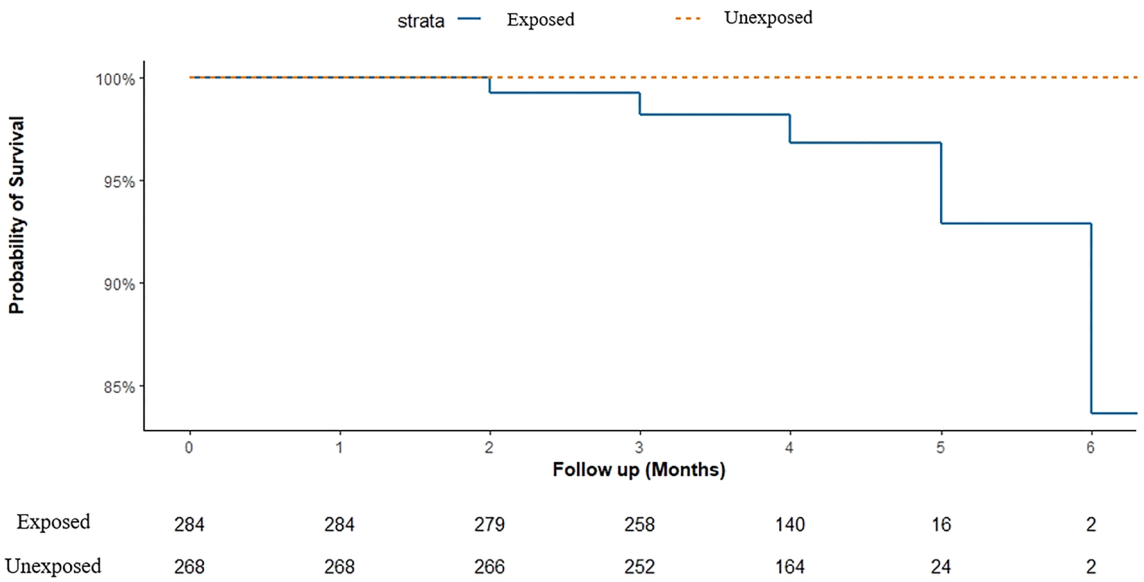

Fig. 3 Kaplan Meier curve showing probability of survival without drug-induced liver injury in PLHIV attending Halibet national referral hospital, 1 March–30 October 2021, Asmara, Eritrea. The figure shows that none of the unexposed group had developed drug-induced liver injury (DILI) during the study period

all patients for 6 months – until they complete their IPT – was a challenge. Taking the short time-to-onset of DILI, namely a median of 52 days [31, 32], the authors decided to follow-up patients for a median of 4 months, and thus, we might have missed cases with delayed time to onset. Rarely, in situations where ALT test was not available, other liver enzymes such as ALP and/or aspartate transaminase (AST) along with total bilirubin were monitored, which might have underestimated the risk. The fact that the study was conducted in one national referral hospital limits generalizability of the results to other treatment sites.

5 Conclusions

Close liver function laboratory monitoring by means of liver function tests, especially during the first 3 months of treatment, is important to safely administer the product. This is because DILI in PLHIV taking IPT was commonly reported, and it was asymptomatic in the majority of the cases despite high levels of ALT. To ensure this, existing laboratory setups need to be further capacitated to minimise challenges associated with IPT implementation.

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Declarations

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Conflict of Interest None declared.

Availability of Data and Material All information gathered is included in the manuscript and additional information can be obtained from the corresponding author via: satiswt@gmail.com or m.russomghebremedhin@erasmusmc.nl.

Ethical Considerations Ethical approval to conduct the study was obtained from the Health Research Ethics and Protocol Review Committee of the Ministry of Health of the State of Eritrea (*reference number: 7-18/2020*). Written consent was obtained from the study participants and all ethical and professional considerations were followed to ensure patient confidentiality. Thus, only anonymized information is reported. The study was performed in accordance with the ethical standards as laid down in the latest version (2013) of the 1964 Declaration of Helsinki.

Consent for participate Prior to enrollment, written consent was obtained from each study participant to take part in the study and their anonymised data to be reported in an international arena.

Consent for publication Not required.

Code availability Not required.

Author Contributions The idea was conceived by MR, KV and BHS. All authors made a significant contribution in the design and follow-up of the study and interpretation of the results. Data was analysed by HGW. Data collection, supervision and data analysis processes were led by MR. The manuscript was drafted by MR and critically reviewed and edited by the rest of the authors. Finally, all the authors gave approval of the manuscript to be published in the journal and agreed to be accountable for all aspects of the work.

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Authors and Affiliations

Mulugeta Russom[1,2,3] · Daniel Y. B. Jeannetot[2] · Araia Berhane[4] · Henok G. Woldu[5,6] · Bruno H. Stricker[7] · Katia M. C. Verhamme[2]

✉ Mulugeta Russom
satiswt@gmail.com; m.russomghebremedhin@erasmusmc.nl

1 Eritrean Pharmacovigilance Centre, National Medicines and Food Administration, Ministry of Health, Asmara, Eritrea

2 Department of Medical Informatics, Erasmus Medical Centre, Rotterdam, The Netherlands

3 European Program for Pharmacovigilance and Pharmacoepidemiology, University of Bordeaux, Bordeaux, France

4 Communicable Disease Control Division, Department of Public Health, Ministry of Health, Asmara, Eritrea

5 The Center for Health Analytics for National and Global Equity, Columbia, MO, USA

6 Bayto AI, Austin, TX, USA

7 Department of Epidemiology, Erasmus Medical Centre, Rotterdam, The Netherlands