

The Impact of Malaria on Liver Enzymes: A Retrospective Cohort Study (2010–2017)

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Background. It is unclear if malaria causes deranged liver enzymes. This has implications both in clinical practice and in research, particularly for antimalarial drug development.

Method. We performed a retrospective cohort study of returning travelers ($n = 4548$) who underwent a malaria test and had enzymes measured within 31 days in Calgary, Canada, from 2010 to 2017. Odds ratios of having an abnormal alkaline phosphatase (ALP), alanine aminotransferases (ALT), aspartate aminotransferases (AST), and total bilirubin (TB) were calculated using multivariable longitudinal analysis with binomial response.

Results. After adjusting for gender, age, and use of hepatotoxic medications, returning travelers testing positive for malaria had higher odds of having an abnormal TB (odds ratio [OR], 12.64; 95% confidence interval [CI], 6.32–25.29; $P < .001$) but not ALP (OR, 0.32; 95% CI, 0.09–1.10; $P = .072$), ALT (OR, 1.01; 95% CI, 0.54–1.89; $P = .978$) or AST (OR, 1.26; 95% CI, 0.22–7.37; $P = .794$), compared with those who tested negative. TB was most likely to be abnormal in the “early” period (day 0–day 3) but then normalized in subsequent intervals. Returning travelers with severe malaria (OR, 2.56; 95% CI, 0.99–6.62; $P = .052$) had borderline increased odds of having an abnormal TB, but malaria species (OR, 0.70; 95% CI, 0.24–2.05; $P = .511$) did not.

Conclusions. In malaria-exposed returning travelers, the TB is abnormal, especially in the early period, but no abnormalities are seen for ALT, AST, or ALP.

Keywords: clinical trials; liver enzymes; malaria.

With 92% of malaria cases and 93% of malaria deaths occurring in sub-Saharan Africa in 2017, the threat of artemisinin resistance spreading to the region is of great public health concern [1]. For malaria control and eradication to be feasible by the target of 2030 [2, 3], there is a necessity to develop novel therapeutic compounds, but no new clinical entities (NCEs) treating blood-stage infections having been developed since artemisinin [4]. The likelihood of artemisinin resistance spreading means that without new medications, significant barriers to control and eradication would remain [5].

As with any drug development, attrition of antimalarial drugs in development pipelines is common and also extremely costly [6]. Characterizing liver enzymes in uncomplicated malaria is becoming increasingly important, as clinical trials of novel

antimalarial NCEs have reported abnormalities in liver enzymes, specifically alanine aminotransferase (ALT) and/or total bilirubin (TB) [7–13]. Regulatory bodies such as the US Food and Drug Administration (FDA) typically use Hy’s law for evidence of liver toxicity in clinical trials, as severe drug-induced liver injury (DILI) tends to occur at a frequency of at least 10% of this rate [14]. Hy’s law sets a danger threshold of $3 \times$ upper limit of normal (\times ULN)–fold elevations of aminotransferases compared with a control arm, with an elevation of serum total bilirubin to $>2 \times$ ULN [14, 15].

The World Health Organization (WHO) guidelines reports that severe malaria can cause multi-organ failure and liver damage, with jaundice being relatively common [16]. In nonsevere malaria, the guidelines state that hyperbilirubinemia is relatively common, and aminotransferase liver enzymes may be elevated up to 10-fold, but signs of liver failure are extremely rare. The extent of abnormalities in aminotransferases are not further characterized by the WHO, and in general, data on nonsevere malaria is more limited. Studies are often heterogeneous, lack control groups, are cross-sectional in nature, or do not control for confounders such as disease severity, making it difficult to draw inferences. Jaundice can occur (possibly due to hemolysis) and raised aminotransferases, but the exact frequency of these results varies depending on the study [17–20].

Furthermore, some approved antimalarial medications can cause mildly deranged liver enzymes [21], leading to further

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difficulties in interpreting trial results. Liver dysfunction can occur due to many currently used antimalarial therapies, either through malaria prophylaxis or treatment. Chloroquine has not been found to cause liver toxicity and appears to be safe in mild liver disease [22]. Sulfadoxine/pyrimethamine is well known to cause hepatic toxicity, including liver granulomas, mixed cholestatic-hepatocellular hepatitis, acute hepatic necrosis, and chronic hepatitis [23–25]. Malarone has been associated with deranged liver enzymes [21], and mefloquine can cause a transient rise in liver enzymes but is not associated with significant hepatotoxicity [26, 27]. Hepatotoxicity with the use of quinine and doxycycline is rare [28, 29]. Artemisinin combination therapies (ACTs) have a good safety profile and generally have replaced other antimalarials due to their efficacy and tolerability. They are known to cause delayed hemolysis and therefore hyperbilirubinemia [30], but some studies have also found that self-limiting, mild rises in aminotransferases can also occur [28, 31].

Historically, the impact of malaria and antimalarials on liver enzymes has been unclear. Drug development trials have shown conflicting results [8–10, 12, 32], but these often enroll small numbers, lack a control group, and are not specifically designed to determine the cause of the liver enzyme abnormalities. This makes it challenging to differentiate whether any liver enzyme changes are due to the malaria itself or the NCEs being tested. However, more recently a number of studies have been conducted that have specifically attempted to address issue [7, 33–35]. A substudy of the West African Network for Clinical Trials of Antimalarial Drugs (WANECAM) phase 3b/4 trials was specifically designed to investigate the prevalence of liver abnormalities among individuals ≥ 6 months in age with uncomplicated malaria; those taking pyronaridine-artesunate (PA) were compared with those taking artemether-lumefantrine (AL) [7]. This study found an ALT rise in 28/1015 (3%) and 7/671 (1%) of PA-treated and AL-treated individuals, respectively, with a TB rise in both groups of $< 1\%$, suggesting that the liver abnormalities are rare in malaria even when taking medications. Silva-Pinto et al. characterized liver enzymes in returning travelers admitted with uncomplicated malaria, finding that individuals taking AL had a higher proportion of abnormal aminotransferases than those taking quinine and doxycycline [33]. A Woodford et al. [34] retrospective cohort study of in-patient returning travelers described a peak TB on the day of admission during the early period, with a raised ALT in the early and late period, but this study lacked a control group and was prone to selection bias, as individuals without serial liver enzyme measurements were excluded. Reuling et al. [35] studied liver enzymes in in-patient returning travelers with uncomplicated malaria and also in controlled human malaria infection (CHMI) studies. Among returning travelers, they reported a peak TB at the time of diagnosis and a prevalence of aminotransferase abnormalities of 69%, far above those

found in other studies. In the CHMI group, whose participants were not returning travelers, similar rises were seen for the aminotransferases but not for TB. This was felt to be due to the lower parasitemia in the CHMI group, leading to less hemolysis. Overall, ALT/aspartate aminotransferases (AST) peaked 2–6 days after initiation of therapy, whereas TB peaked before antimalarial administration. However, there are differences between naturally acquired malaria and CHMI [36], and the time course of rises makes it difficult to differentiate between the effects of malaria and its treatments.

To characterize liver enzymes in malaria, a retrospective cohort study was designed to obtain longitudinal data on liver enzymes in returning travelers. The primary objective was to ascertain the effect of malaria on aminotransferases and TB, with the secondary objective being to ascertain the time frame when any changes in liver enzymes occur from the initial malaria test.

METHODS

Study Design and Ethics

Symptomatic returning travelers were eligible for enrollment if they presented for malaria testing due to in the period 2010–2017 at Calgary Laboratory Services (CLS) in Calgary and had at least 1 liver enzyme tested within 31 days of the index malaria test. All eligible individuals were enrolled for the liver enzyme analyses, but due to the large number of non-malaria-exposed participants, individuals were randomly selected to be enrolled for the epidemiological analyses. Individuals had epidemiological data recorded on a patient information sheet by the clinician doing the malaria testing. A chart review was undertaken that obtained liver enzymes, epidemiological data, disease severity, and out-patient medication data. Malaria severity was defined in line with WHO guidelines [16]. Out-patient prescriptions for the 6 months before malaria testing were obtained and reviewed for evidence of hepatotoxic medications, as defined by the National Institutes of Health LiverTox database [28]. The liver enzymes recorded were alkaline phosphatase (ALP), ALT, AST, and TB. Ethical approval was obtained from the University of Calgary Ethics Board (Ethics ID: REB15-1160).

Outcomes

The primary outcome was to determine whether malaria leads to increased ALT and TB within 31 days of malaria testing. These liver enzymes were chosen because they were felt to be the most commonly used and relevant markers of liver injury. AST is not regularly tested at CLS, and ALP is more typically raised in obstructive causes of deranged liver enzymes. The secondary objective was to determine the time period when any abnormalities in ALT and TB occur, starting from the initial blood draw. Time periods from index malaria test were split in line with previous literature into an early period: 0–3 days; intermediate period: 4–11 days; and a late period: 12–31 days [34].

Malaria and Liver Enzyme Testing

Each individual eligible for enrollment was tested according to standard operating procedure at the time: 3 Giemsa-stained thick and thin peripheral blood smears at least 6 to 8 hours apart and rapid diagnostic tests (RDTs) (BinaxNOW Malaria, Alere, Waltham, MA). Malaria species were identified by microscopy. Liver enzyme testing occurred as part of routine clinical testing on whole-blood specimens, with upper limits of normal used in clinical practice [37]. All liver enzyme measurements were converted into multiples of the age- and gender-specific upper limit of normal, termed \times ULN. This accounted for the differing upper limits of normal for different genders and age groups. The results for the \times ULN values were highly positively skewed, so they were dichotomized into either normal or abnormal values.

Statistical Analysis

First, the impact of epidemiological risk factors on malaria test results was analyzed. Univariable statistical analysis of epidemiological dichotomous variables was performed using chi-square or Fisher exact statistical tests with *t* tests used for continuous variables, then by multivariable logistic regression. Results for the 4 liver enzymes were analyzed separately. To allow for the generation of results for the univariable analysis, if a participant

had multiple values taken within the same time period, the mean value was used. The prevalence of Hy's law was calculated according to these values. For the multivariable analysis, results were obtained over a 31-day period, so they were treated as longitudinal data. Due to evidence of clustering (Supplementary Table 5 and Supplementary Figure 1), logistic regression of binomial longitudinal panel data was performed with robust standard errors and adjustments for serial correlation. The time period was included with early, intermediate, and late values to ascertain their influence on liver enzyme results. Analysis was performed on liver enzymes individually, with missing data excluded from the model. Further details of the methods can be found in the Supplementary Data.

RESULTS

Prevalence of Epidemiological Characteristics With Malaria

As shown in Figure 1, initial analysis revealed a total 17 207 liver enzyme results from 4548 individuals receiving a malaria test, with a 241/4548 (5.3%) malaria positivity rate. The univariable analysis shown in Table 1 revealed that malaria-positive and -negative individuals were of a similar mean age (malaria positive, 33.30 years; 95% confidence interval [CI],

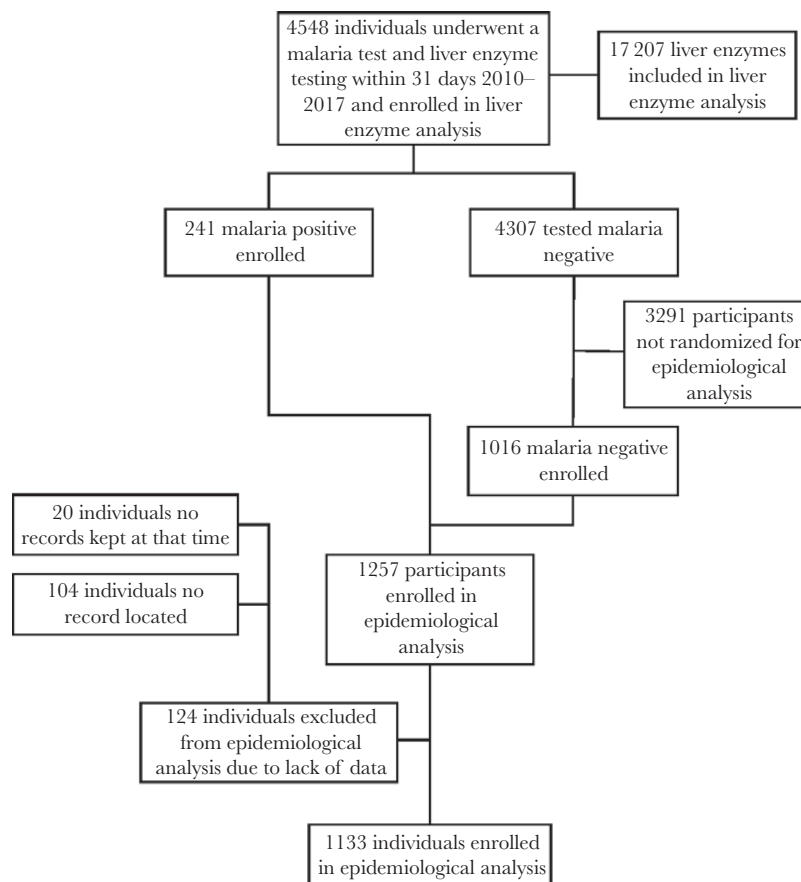


Figure 1. Flow diagram of study.

Table 1. Demographics of Study Participants by Malaria Test Result

	Total	Malaria Positive	Malaria Negative	PValue ^a
No. of patients	1257	241	1016	
Age, mean (95% CI), y	35.35 (34.28–36.42)	33.30 (31.11–35.49)	35.84 (34.62–37.06)	.067 ^b
Male, No. (%)	694 (55.21)	156 (64.73)	538 (52.95)	.001 ^c
Parasitemia, median (IQR)		0.2 (1.1)		
Malaria species, No. (%)				
<i>P. falciparum</i>		151 (62.66)		
<i>P. falciparum/P. ovale</i>		5 (2.07)		
<i>P. malariae</i>		1 (0.41)		
<i>P. ovale</i>		16 (6.64)		
<i>P. vivax</i>		56 (23.24)		
<i>P. vivax/P. ovale</i>		1 (0.41)		
Not documented		11 (4.56)		
Malaria severity (n = 192), No. (%)		44 (22.92)		
Pretravel advice (n = 867), ^d No. (%)	297/867 (34.26)	39/161 (24.22)	258/706 (36.54)	.003 ^c
Malaria prophylaxis taken (n = 495), ^d No. (%)	119/495 (24.0)	18/104 (17.31)	101/391 (25.83)	.071 ^c
Reason for test (n = 1133), ^d No. (%)				
Business	96/1133 (8.47)	13/199 (6.53)	83/934 (8.89)	.279 ^c
Visiting friends/relatives	485/1133 (42.81)	104/199 (52.26)	381/934 (40.79)	.003 ^c
New immigrant	132/1133 (11.65)	40/199 (20.10)	92/934 (9.85)	<.001 ^c
Tourism	295/1133 (26.04)	17/199 (8.54)	278/934 (29.76)	<.001 ^c
Visitor to Canada	16/1133 (1.41)	10/199 (5.03)	6/934 (0.64)	<.001 ^c
Not recorded	109/1133 (9.62)	15/199 (7.54)	94/934 (10.06)	.272 ^c
Continent visited (n = 1073), ^d No. (%)				
Non-Africa	631/1073 (58.81)	36/194 (18.56)	595/879 (67.69)	
Africa	442/1073 (41.19)	158/194 (81.44)	284/879 (32.31)	<.001 ^c
Symptoms ^e (n = 1054), ^d No. (%)				
Fever	889/1054 (84.35)	162/181 (89.50)	727/873 (83.28)	.036 ^c
Night sweats	391/1054 (37.10)	73/181 (40.33)	318/872 (36.43)	.322 ^c
Headache	561/1054 (53.23)	116/181 (64.09)	445/873 (50.97)	.001 ^c
Cough	317/1054 (30.08)	45/181 (24.86)	272/873 (31.16)	.093 ^c
Sore throat	243/1054 (23.06)	21/185 (11.60)	222/873 (25.43)	<.001 ^c
Myalgia	390/1054 (37.00)	78/181 (43.09)	312/873 (35.74)	.062 ^c
Other	298/1054 (28.27)	48/181 (26.52)	250/873 (28.64)	.565 ^c
Hepatotoxic medication, No. (%)	588 (46.78)	105 (43.57)	483 (47.54)	.267 ^c

Abbreviations: CI, confidence interval; IQR, interquartile range.

^aP value compares malaria-positive with malaria-negative group, missing values excluded.

^bT test of means.

^cChi-square test.

^dNo. less than grand total due to missing data, percentages calculated excluding missing data, and statistical tests excluding missing data.

^eTotal does not add to 100 as participants reported more than 1 symptom.

31.11–35.49 years; malaria negative, 35.84 years; 95% CI, 34.62–37.06 years), but there were statistically more males ($P = .001$) in the malaria-positive group (156/241, 64.7%) compared with the malaria-negative group (538/1016, 53.0%). Among the malaria-positive group, the most common species responsible was *P. falciparum* (156/241, 64.7%), with 22.9% of the malaria-positive individuals suffering from severe malaria. For the multivariable logistic regression, there was evidence of correlation (Supplementary Table 1) between pretravel advice and the use of antimalarial prophylaxis, so only antimalarial prophylaxis was included alongside the other variables. As seen in Figure 2, returning travelers who were male (OR, 2.26; 95% CI, 1.23–4.15) and those who had visited Africa (OR, 19.47; 95%

CI, 9.73–38.95) had statistically higher odds of testing positive for malaria, whereas those taking antimalarial prophylaxis had lower odds (OR, 0.20; 95% CI, 0.10–0.41). Individuals with a headache (OR, 2.27; 95% CI, 1.20–4.30) had statistically higher odds of being malaria positive, whereas those presenting with a sore throat (OR, 0.13; 95% CI, 0.05–0.36) had lower odds.

Impact of Malaria on Liver Enzymes

A total of 17,207 individual liver enzyme measurements were performed in the 31 days after the first malaria test. In the early period, 221/241 (91.7%) of malaria-positive individuals had an ALT measured, compared with 3816/4037 (94.5%) of malaria-negative individuals. However, the percentage of

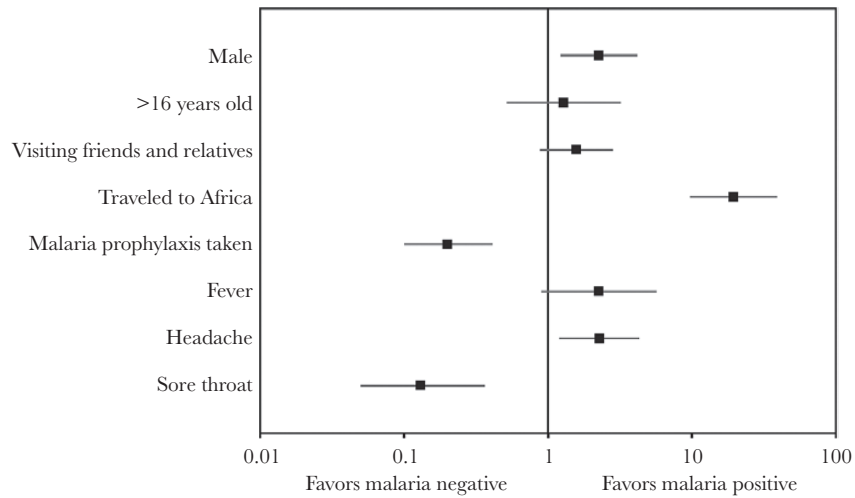


Figure 2. Logistic regression analysis of epidemiological risk factors.

malaria-positive individuals with ALT measurements in the intermediate and late periods was 11.6% and 11.2%, respectively, with for 11.8% and 15.2% for negative individuals. This demonstrates how there is an increased testing rate in the early period compared with the intermediate and late periods. For TB, the respective values for malaria-positive individuals are 72.6%, 11.6%, and 10.0%, with 38.7%, 8.2%, and 9.9% for negative individuals, meaning a greater proportion of malaria-positive individuals had a TB measured. For ALP and AST, a similar pattern of testing was noted, with the vast proportion of tests performed in the early period. The median values in each time period for all liver enzymes are shown in [Supplementary Table 4](#). This analysis suggested that the median TB of malaria-exposed individuals may be higher than for malaria-unexposed individuals, but no difference was found for ALP, ALT, or AST. The proportion of individuals with abnormal liver enzymes is shown in [Table 2](#). The proportion of individuals with abnormal ALT was not statistically different in any period, with the proportion abnormal in the early period being 42/221 (19.0%) in the malaria-exposed group and 624/3816 (16.4%)

in the unexposed group. For TB in the early period, the proportion with an abnormal result was 48/175 (27.4%) in the exposed group, compared with 129/1665 (7.8%) in the unexposed group, with no difference in the intermediate and late periods. Malaria-negative individuals were more likely to have an abnormal ALP in the early period than malaria-positive individuals ($P = .049$), but no other statistically significant difference was found in other time frames. For AST, no statistically significant difference was found in any time period. Univariable analyses of severity of malaria, species, and use of hepatotoxic medications ([Supplementary Table 6](#)) showed that individuals with severe malaria were statistically more likely to have an abnormal TB than those with nonsevere malaria in the early period. Use of hepatotoxic medication appeared to influence ALP in the early period ($P = .001$) and ALT in the intermediate period ($P = .039$), but no other associations had a statistically significant difference, and results were not consistent across all time periods.

In terms of Hy's law ([Table 3](#)), there was no difference in the proportion of malaria-positive individuals vs malaria-negative

Table 2. Proportion of Abnormal Liver Enzymes by Malaria Result

Time Period	ALP, No. (%)			ALT, No. (%)			AST, No. (%)			TB, No. (%)		
	Early (n = 3098)	Intermediate (n = 431)	Late (n = 555)	Early (n = 4037)	Intermediate (n = 538)	Late (n = 682)	Early (n = 640)	Intermediate (n = 118)	Late (n = 156)	Early (n = 1840)	Intermediate (n = 381)	Late (n = 450)
Malaria result												
Negative	197/2888 (6.82)	114/409 (27.87)	105/532 (19.74)	624/3816 (16.35)	241/510 (47.25)	168/655 (25.65)	166/602 (27.57)	65/111 (58.56)	57/153 (37.25)	129/1665 (7.75)	63/353 (17.85)	57/426 (13.38)
Positive	7/210 (3.33)	2/22 (9.09)	1/23 (4.35)	42/221 (19.00)	12/28 (42.86)	5/27 (18.52)	12/38 (31.58)	4/7 (57.14)	2/3 (66.67)	48/175 (27.43)	2/28 (7.14)	4/24 (16.67)
P	.049	.080	.099	.302	.650	.404	.593	1.000	.557	<.001	.195	.551

If multiple tests done on participants within same time frame, mean of results used. All by chi-square or Fisher exact; statistically significant differences shown in bold. Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferases; AST, aspartate aminotransferases; TB, total bilirubin.

Table 3. Proportion of Participants That Meet the Criteria for Hy's Law Depending on Malaria Test Result for the Early, Intermediate, and Late Time Periods

		Early Period		Intermediate Period		Late Period	
		Malaria Result		Malaria Result		Malaria Result	
		Negative (n = 1581)	Positive (n = 166)	Negative (n = 336)	Positive (n = 18)	Negative (n = 409)	Positive (n = 18)
Hy's law	No	1549 (97.98)	162 (97.59)	316 (94.05)	17 (94.44)	398 (97.31)	17 (94.44)
	Yes	32 (2.02)	4 (2.41)	20 (5.95)	1 (5.56)	11 (2.69)	1 (5.56)
<i>P</i> ^b		.771		1.000		.408	

^aCriteria for Hy's law based on mean of individual participants' results within any given time period.

^bFisher exact test.

individuals meeting the criteria in the early (2.4%, 2.0%; *P* = .771), intermediate (5.6%, 6.0%; *P* = 1.000), or late (5.6%, 2.7%; *P* = .408) periods. Of the 4 malaria-positive individuals meeting Hy's law, 1 individual had severe *P. falciparum* and subsequently succumbed to the illness, 1 had nonsevere *P. vivax* and had taken multiple illicit drugs, 1 had nonsevere *P. ovale* with G6PD deficiency, and the final individual had severe *P. falciparum* with an acute hepatitis A co-infection.

For the multivariable analysis, ALP, ALT, AST, and TB were analyzed as the dependent variables separately to assess the result for individual liver enzymes. To allow for longitudinal logistic regression to be performed, only the first liver enzyme result performed on an individual on any given day was taken, leading to the exclusion of 5 ALP, 6 ALT, 0 AST, and 5 TB results. This left 5412 ALP, 6843 ALT, 1075 AST, and 3861 TB measurements, respectively, included in the analysis. For ALT, 6843 observations were performed on 1271 individuals, with a mean number of observations per individual of 1.5. For TB, 3861 observations were performed on 2186 individuals, leading to mean of 1.8 observations per individual. Gender (*P* = .451), age (*P* = .337), and hepatotoxic medication (*P* = .186) appeared to have no effect on the odds of having an abnormal ALT result (Table 4). In addition, malaria positivity also appeared to have no effect (OR, 1.01; 95% CI, 0.54–1.89; *P* = .978). Using the early period as a reference, ALT results from the intermediate

period had significantly higher odds of being abnormal (OR, 2.57; 95% CI, 1.68–3.92; *P* < .001), but the late period did not (OR, 0.67; 95% CI, 0.40–1.14; *P* = .139). For TB, males had higher odds of an abnormal result (OR, 5.72; 95% CI, 2.85–11.49; *P* < .001); adults had lower odds than children (OR, 0.43; 95% CI, 0.21–0.91; *P* = .026), but hepatotoxic medication (*P* = .751) did not. Individuals testing positive for malaria had significantly higher odds of having an abnormal TB result (OR, 12.64; 95% CI, 6.32–25.29; *P* < .001). Compared with the early period, TB measured in the intermediate (OR, 0.58; 95% CI, 0.33–1.04; *P* = .070) and late periods (OR, 0.72; 95% CI, 0.37–1.39; *P* = .324) had lower odds of being abnormal, but these were not significant. The malaria result had no effect on the odds of having an abnormal ALP (OR, 0.32; 95% CI, 0.09–1.10; *P* = .072) or AST (OR, 1.26; 95% CI, 0.22–7.37; *P* = .794). For the time periods, after adjusting for the other variables; the results for ALP and AST followed a similar pattern as ALT, with values measured in the intermediate period having higher odds of being abnormal than in the early period. Examining malaria-positive results only (Supplementary Table 7), disease severity was borderline statistically significant for abnormal TB (OR, 2.56; 95% CI, 0.99–6.62; *P* = .052), but having *P. falciparum* species (OR, 0.70; 95% CI, 0.24–2.05; *P* = .511) did not appear to significantly increase the odds of having an abnormal TB. No statistically significant difference was found for ALT.

Table 4. Odds Ratios and Their Corresponding 95% CIs and P Values for Selected Determinants of Having Abnormal Liver Enzymes

Liver Assay	ALP		ALT		AST		Bilirubin	
No. of observations	5412		6843		1075		3861	
No. of individuals	3443		4417		806		2186	
Mean obs per individual	1.6		1.5		1.3		1.8	
	Odds Ratio	<i>P</i> Value	Odds Ratio	<i>P</i> Value	Odds Ratio	<i>P</i> Value	Odds Ratio	<i>P</i> Value
Gender (male)	0.75 (0.47–1.21)	.242	0.89 (0.66–1.21)	.451	0.60 (0.26–1.36)	.220	5.72 (2.85–11.49)	<.001
Age (adult)	23.30 (7.30–74.40)	<.001	1.22 (0.81–1.83)	.337	2.82 (1.06–7.46)	.037	0.43 (0.21–0.91)	.026
Hepatotoxic medication (taken)	1.42 (0.89–2.28)	.140	1.23 (0.90–1.68)	.186	1.45 (0.65–3.28)	.367	0.91 (0.50–1.63)	.751
Malaria result (positive)	0.32 (0.09–1.10)	.072	1.01 (0.54–1.89)	.978	1.26 (0.22–7.37)	.794	12.64 (6.32–25.29)	<.001
Early time period (reference)	1.00		1.00		1.00		1.00	
Intermediate time period	4.23 (2.66–6.72)	<.001	2.57 (1.68–3.92)	<.001	3.56 (1.26–10.04)	.016	0.58 (0.33–1.04)	.070
Late time period	2.28 (1.25–4.16)	.007	0.67 (0.40–1.14)	.139	0.61 (0.18–2.03)	.419	0.72 (0.37–1.39)	.324

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferases; AST, aspartate aminotransferases.

DISCUSSION

The primary objectives of this study were to investigate whether malaria could lead to abnormal liver enzymes in returning travelers and, if so, to ascertain the time course of any changes. In the multivariable analysis, males had increased odds of having a higher TB but not ALT when compared with females. As described in a number of different studies, males appear to have a higher baseline TB compared with females [38, 39]. Our laboratory uses an identical upper limit of normal for both sexes, so this most likely introduced some misclassification bias for abnormal TB and therefore an overestimation of the odds ratio. Interestingly, those taking hepatotoxic medication were not at higher risk of having an abnormal ALT or TB. However, these medications were being taken in the 6 months before the index malaria test, so individuals were likely to be relatively stable on their medications. Malaria-positive individuals were more likely to have an abnormal TB, with rises in this group most likely to occur during the early period. This is in line with previous literature in returning travelers [34, 35] and appears to fit with a raised TB occurring with malaria, which then begins to return to normal as the individual is treated. As reviewed by Anand et al., hyperbilirubinemia can be conjugated or unconjugated, and the causes are multifactorial, including intravascular hemolysis, disseminated intravascular coagulation, drugs, hepatitis, and G6PD deficiency [20]. The results from this study, along with the biologically plausible explanation, appear to confirm results from other studies that malaria causes raised TB. In contrast to the TB, no increased odds of abnormal ALT results with malaria positivity were found. Other studies have described a raised ALT in returning travelers [34, 35], but these studies lacked a control group and may be prone to selection bias. Anand et al. reported histological changes in the liver, including centrilobular necrosis, Kupffer cell hyperplasia, hemozoin deposition, lymphocytic infiltration, and steatosis [20]. However, evidence for this comes from studies with small sample sizes and specimens from fatal cases. After adjusting for a multivariable analysis, given that a strong association was seen with TB, one would have expected to find an association, should one exist. In contrast to Woodford et al. [34], even when examining ALT results for malaria-positive individuals only, there was no statistically significant difference seen across the 3 time periods, but the sample size was small in the intermediate and late time periods.

A key strength of this study is the use of a multivariable analysis, which has not been done by other studies. Other studies of returning travelers typically exclude those without serial liver enzyme measurements or do not adjust for clustering, leaving them vulnerable to selection bias and therefore overestimating the prevalence of liver enzyme abnormalities. Symptomatic returning travelers are obviously a different group than individuals enrolled in CHMI trials, with returning travelers being more likely to have been exposed to many novel pathogens, to have

taken new medications, or to have been exposed to blood-borne viruses. The use of a control group allows us to make an accurate assessment of the impact of malaria in returning travelers, because these confounding factors are similar across the 2 groups. Furthermore, compared with other studies, the sample size is quite large, so it should be powered to detect any difference in ALT.

One potential criticism of this study is that the malaria group is not compared with a completely “normal” group, but instead one that is symptomatic and often presenting to the hospital. This was deliberate, as we attempted to control for confounding factors in returning travelers and reduce sources of bias. The nonmalaria group will have been exposed to an array of different diagnoses, so we must be cautious when interpreting these results. This limits the generalizability of this study with respect to antimalarial drug development, as this control group does not reflect a population in which drug development occurs, making comparisons challenging. Furthermore, as these are real-life patients and clinical specimens, the liver enzymes available are those from routine clinical care. Consequently, only total bilirubin, and not unconjugated bilirubin, was available for analysis, making it challenging to differentiate the effects of malaria, hemolysis, and genetic conditions such as Gilbert’s syndrome on bilirubin results. Our statistical analysis of this may also be criticized. Although this is a retrospective cohort study, we have chosen to use logistic regression as our outcome measure of choice. This may be considered controversial because risk ratio is traditionally used as an outcome measure for cohort studies.

This is the first study to utilize multivariate regression analysis in a longitudinal study specifically examining liver enzymes in malaria. In this comparison of malaria-exposed and nonexposed returning travelers, the odds of having an abnormal TB were significantly higher in the malaria-exposed group in the early period compared with the nonexposed group, but no difference was seen for ALT, AST, or ALP. Further prospective studies are required to clarify liver enzymes in the setting of drug development.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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