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Prevalence and time course of elevated serum levels of liver enzymes in otherwise healthy Thai infants with breast milk jaundice: a cohort study

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Abstract

Background: Neonatal jaundice and elevated levels of liver enzymes are found in infants with breast milk jaundice (BMJ).
Objectives: To determine the prevalence and duration of elevated serum levels of liver enzymes in Thai infants with BMJ.
Methods: We conducted a prospective study of Thai infants with BMJ, excluding those with pathological causes of jaundice. We measured the serum levels of total bilirubin (TB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and γ-glutamyl transferase (GGT); followed infants with elevated levels; and estimated the time for levels to become normal using Kaplan–Meier analysis.
Results: We included 42 infants (median age: 17.5 days) with BMJ, and elevated serum levels of at least 1 enzyme were found in 27 (64%) infants. We excluded 4 (10%) infants because they did not continue to be exclusively breastfed, 17 (40%) were lost to follow-up, and 21 (50%) completed the study. We found that 19 (45%) of the 42 infants had elevated GGT, 11 (26%) had elevated ALT, and 9 (21%) each had elevated AST and ALP levels. The median time for enzyme levels to normalize was 291 days (95% confidence interval [CI], 109.8 to 472.2) for ALT, 240 days (95% CI, 139.0 to 340.9) for AST, 184 days (95% CI, 4.4 to 363.6) for ALP, 120 days (95% CI, 74.6 to 164.5) for TB, and 63 days (95% CI, 61.44 to 64.6) for GGT. Infants were otherwise healthy during the follow-up.
Conclusion: The prevalence of elevated serum levels of liver enzymes in Thai infants was unexpectedly high, but the levels became normal spontaneously despite continued breastfeeding, which endorses a "watchful waiting" strategy in managing asymptomatic infants with BMJ.

Keywords: breastfeeding; hyperbilirubinemia, neonatal; jaundice, neonatal; liver function tests; Thai

Unconjugated hyperbilirubinemia is a common finding in about 60% of term and late preterm newborns, and it is defined as physiological jaundice [1]. There are 2 clinical entities associated with breastfeeding and jaundice. The first is relative dehydration due to insufficient breast milk intake, which promotes exaggerated enterohepatic circulation of bilirubin [2]. Breastfeeding jaundice may occur during the first postnatal day, but it usually resolves before 10 days of life [1]. The second clinical entity, breast milk jaundice (BMJ), usually occurs later, in the late first or second week of life and continues until 3 months of life before resolving spontaneously [1, 2]. BMJ is considered a normal physiological condition, whose true mechanism is poorly understood [3]. The incidence of BMJ in the first 2–3 weeks of life may be as high as 34% [4]. Although breastfeeding interruption was considered a diagnostic and therapeutic intervention for this condition in

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the past, current data do not support interrupting the breastfeeding of infants with suspected BMJ. Instead, a thorough investigation is recommended to exclude pathological causes of prolonged unconjugated hyperbilirubinemia in suspected or symptomatic cases [2, 3, 5].

The American Academy of Pediatrics [5] and the Ministry of Public Health in Thailand recommend breastfeeding throughout the first 6 months of life. This might result in an increasing number of infants with hyperbilirubinemia because breastfeeding has greater potential to cause hyperbilirubinemia in infants than formula feeding [6–8]. Most infants with BMJ have at least 1 item of abnormal liver enzyme level [1, 6, 8–11]. To our knowledge, until now, no study to determine the prevalence of abnormal levels of liver enzymes in Thai infants with BMJ or the time needed for all abnormal liver enzyme levels to normalize has been reported. The objectives of the present study were to determine the prevalence and the duration of abnormal levels of liver enzymes in otherwise-healthy asymptomatic Thai infants with BMJ.

Methods

Study design and setting

After approval by the Institutional Review Board (IRB) of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (certificate of approval No. 615/2017; IRB No. 231/60) and written informed consent was provided by the parents of the infants included in the present study, we conducted a prospective cohort study of infants who attended the Department of Pediatrics, King Chulalongkorn Memorial Hospital (KCMH), Bangkok, Thailand, from October 2017 to January 2019, to determine the prevalence of abnormal liver biochemistry in the serum of Thai infants with BMJ and followed them until normalization of all levels. The study was conducted in accordance with the contemporary revision of the Declaration of Helsinki, The Belmont Report, the Council for International Organizations of Medical Sciences Guidelines, and International Conference on Harmonization in Good Clinical Practice. KCMH is a public general and tertiary referral teaching hospital in Bangkok, Thailand, with an inpatient capacity of around 1,500 beds, making it one of the largest hospitals in Thailand.

Participants

We screened 146 neonates with visible jaundice. We excluded those with any possible pathological cause of prolonged hyperbilirubinemia [comprising preterm infants (n = 19); infants with glucose-6-phosphate dehydrogenase deficiency (n = 21), hemolysis (n = 12), hematoma (n = 15), and infection (n = 2)]; infants whose parents refused enrolment of their infants in the study (n = 29); and others (n = 6). We enrolled 42 infants with BMJ, defined as having a total bilirubin (TB) level >1.2 mg/dL [12] for >2 weeks. All infants were otherwise healthy, characterized by normal weight gain, normal stool and urine output, normal physical examination, and no signs or symptoms of underlying pathology [2].

The infants were divided into 2 groups, namely, those with "normal liver enzyme levels" and those with "elevated liver enzyme levels", according to the serum level of liver enzymes. In the group with normal liver enzyme levels, blood samples were collected at the first presentation and at the age of 2 months. The serum measurements were repeated at the age of 2 months because of the late onset of elevated ALT at the age of 4 weeks [1] and because this age follows an unrelated immunization schedule. In the group with elevated liver enzyme levels, blood samples were collected at the first presentation and periodically during the follow-up for 2–12 weeks on a case-by-case basis depending on the degree of abnormal liver biochemistry until all the serum levels of liver enzymes became normal. At the age of 2 months, any infants in the group with normal liver enzyme levels who had at least 1 elevated liver enzyme level were transferred to the group with elevated liver enzyme levels and followed until all liver enzyme levels became normal. On every follow-up occasion, infants were examined for possible pathological diseases, and we monitored their growth and development. Meanwhile, we excluded any infants whose breastfeeding was supplemented with formula.

Variables

In all infants, we measured the serum levels of TB, direct bilirubin (DB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and γ-glutamyl transferase (GGT) using a commercial test kit (Alinity ci- Series Clinical Chemistry Instrument, Abbott). Normal values of liver biochemistry for age were referenced from the *Nelson Textbook of Pediatrics* [12] and *The Harriet Lane Handbook* [13]. The level of an enzyme was defined as elevated when it was more than the upper limit of the reference value for age.

Statistical analyses

Categorical data such as sex, gestational age, birth weight, and delivery mode are presented as number and percentage.

Continuous data are expressed as mean and standard deviation (SD) or median and range. An independent *t* test was used to test differences between continuous data, and a Kaplan–Meier survival estimate was used to determine the duration of elevated liver enzyme and TB levels. All statistical analyses were conducted using IBM SPSS Statistics for Windows (version 22). Differences with $P < 0.05$ were considered significant.

Results

Participants and demographic data

Of the 42 infants included in the present study, 15 (36%) ultimately had normal liver biochemistry. At the age of 2 months, of the 20 infants originally with normal liver enzyme levels, 5 ultimately had at least 1 elevated liver enzyme level; these infants were then reclassified into the group with elevated liver enzyme levels. Meanwhile, 1 infant from the group with normal liver enzyme levels and 3 infants from the group with elevated liver enzyme levels were excluded from the study because their feeding was supplemented with formula. At the end of the study, with a median follow-up period of 64 days (range: 39–365 days), 21 infants (50%) completed the study (**Figure 1**).

The median age of the infants was 17.5 (range: 7–64) days. The ratio of infant boys to girls was 1.6:1. The majority of infants (88%) had normal birth weight (**Table 1**).

Prevalence of elevated liver enzymes

Of the initial 42 infants, 27 (64%) had elevated levels of liver enzymes (**Figure 1**).

At the first presentation

The most frequently elevated liver enzyme was GGT in 19 infants (45%), followed by ALT in 11 (26%), and AST and ALP in 9 each (21% each). A large proportion of infants (8/11) had a normal level of ALT at the first presentation, which became elevated when the infants were 2 months of age.

At the end of the study

Serum levels of liver enzymes measured in the infants in the group with elevated liver enzyme levels who completed the study and the enzyme levels in those who were lost to follow-up were comparable (**Table 2**).

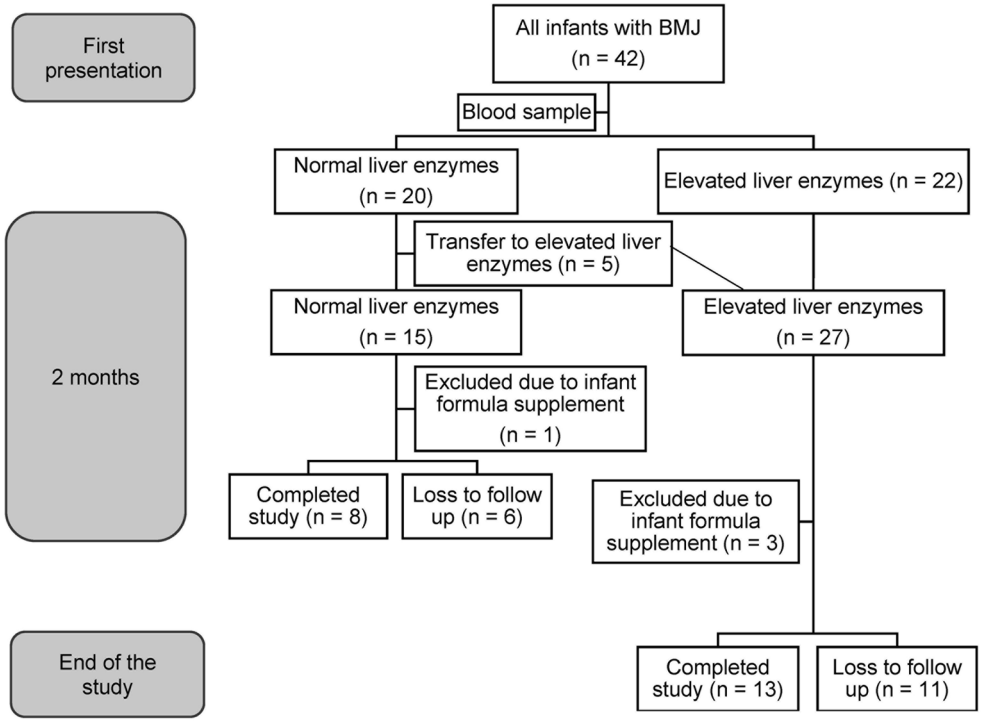

Figure 1. Flow diagram showing the number of enrolled infants at the first presentation and at the end of the study.

Table 1. Demographic variables and differences between groups of infants with normal and elevated liver enzymes at the first presentation

Variable	Normal liver enzyme levels (n = 15)	Elevated liver enzyme levels (n = 27)	*P*
Sex			
Male	8	18	0.39
Female	7	9	
Gestational age (weeks)			
37	2	8	0.59
38	9	12	
39	3	3	
40	1	3	
>40	0	1	
Birth weight (g)			
<2500	1	0	0.43
2501–3000	4	11	
3001–3500	9	13	
>3500	1	3	
Mode of delivery			
Normal delivery	6	13	0.61
Cesarean section	9	14	
Liver enzyme (mean ± standard deviation)			
AST (U/L)	24.0–69.0 (35.6 ± 11.3)	22.0–184.0 (50.4 ± 33.1)	0.103
ALT (U/L)	10.0–33.0 (17.4 ± 6.2)	10.0–56.0 (25.2 ± 13.2)	0.013*
ALP (U/L)	139.0–479.0 (262.5 ± 8.0)	131.0–546.0 (320.8 ± 105.8)	0.096
GGT (U/L)	57.0–124.0 (94.0 ± 23.6)	55.0–352.0 (204.8 ± 75.8)	<0.001*

AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase, and GGT, γ-glutamyl transferase. *$P < 0.05$

Duration of elevated liver enzyme levels

In the group with elevated serum levels of liver enzymes, the median time for the elevated levels to normalize were as shown in **Figure 2**.

Duration of hyperbilirubinemia

If the serum levels of all liver enzymes measured became normal, we did not continue to follow infants until their serum level of TB was <1.2 mg/dL [13] because the aim of the present study was primarily to determine the duration of abnormal liver enzyme levels.

When we compared the TB level between the 2 groups of infants at the age of 2 months, in the 35 infants remaining in the study (loss of 6 to follow-up, exclusion of 1 because of formula supplementation, and 4 due to phenobarbital exposure, which diminishes TB level), we found that the TB level tended to be slightly higher in the group with elevated serum levels of liver enzymes, but this was not significantly different ($P = 0.09$; **Table 3**).

In infants with elevated serum levels of liver enzymes, the median time for serum TB levels to normalize was 120 days (95% CI, 74.6 to 164.5; **Figure 2E**).

Discussion

In the present study, the prevalence of elevated serum levels of liver enzymes in Thai infants with BMJ was 64%, which is much higher than that reported for infants in other studies (36.4% [8] and 31.0% [14]). Poddighe et al. [1] found that levels of serum TB and all liver enzymes measured in the present study, including AST, ALT, and GGT, were abnormal, even though they were not abnormal at the first presentation.

Table 2. Comparison of serum levels of liver enzymes between participants who completed the study and were lost to follow up in the group with elevated liver enzyme levels

Group	Range (mean ± standard deviation)			
	GGT (U/L)	ALT (U/L)	AST (U/L)	ALP (U/L)
Completed	55.0–341.0 (196.7 ± 90.7)	13.0–55.0 (27.5 ± 12.3)	27.0–109.0 (51.8 ± 21.9)	137.0–513.0 (319.7 ± 105.5)
Lost to follow up	156.0–352.0 (212.2 ± 62.9)	10.0–56.0 (23.1 ± 14.2)	22.0–184.0 (49.1 ± 41.8)	131.0–546.0 (321.8 ± 110.4)
P	0.66	0.39	0.84	0.96

AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase, and GGT, γ-glutamyl transferase.

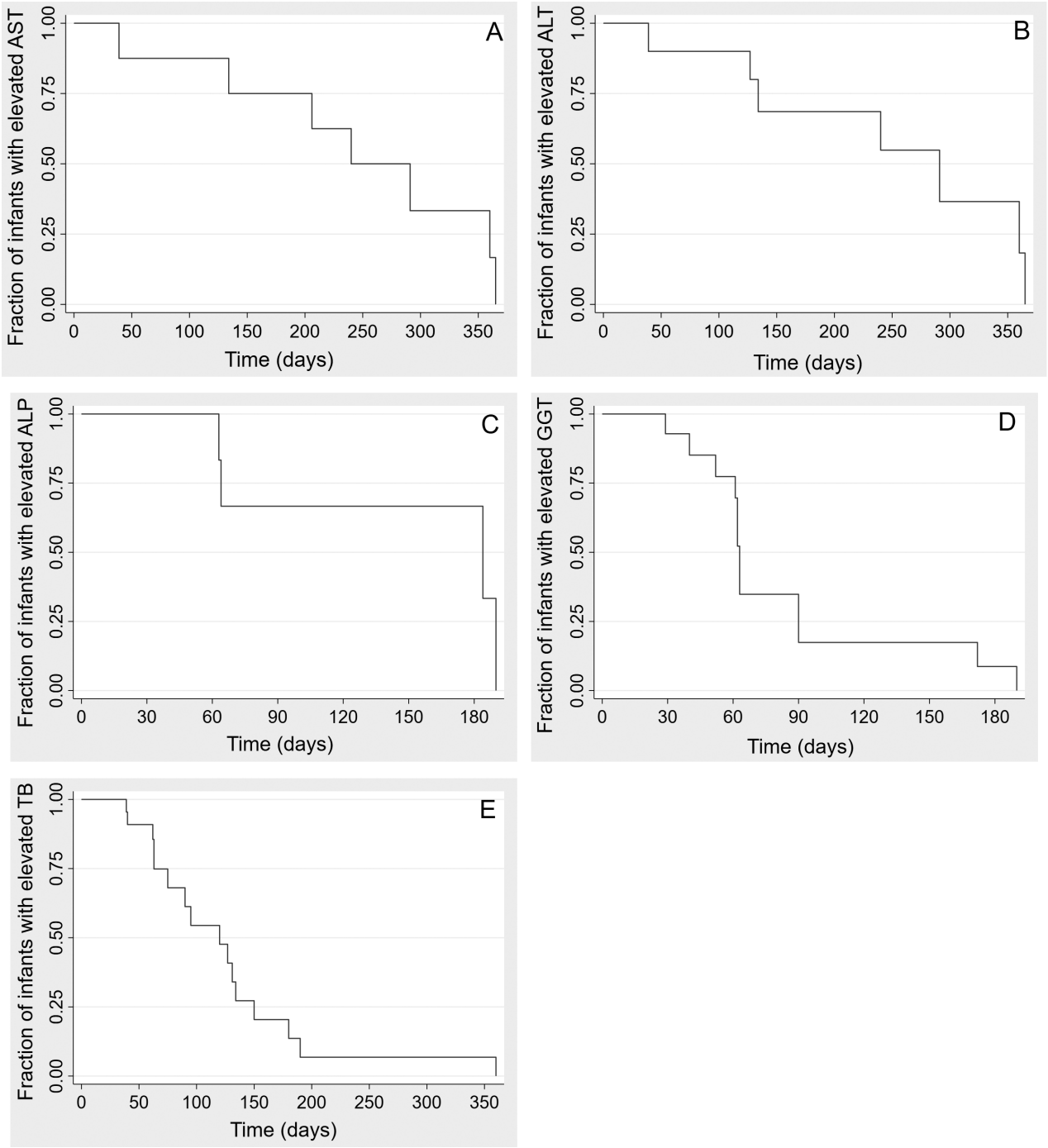

Figure 2. Kaplan–Meier survival curves to estimate the median time for elevated serum levels of liver enzymes and total bilirubin (TB) to normalize. (**A**) Aspartate aminotransferase (AST): 240 days (95% confidence interval [CI], 139.0 to 340.9); (**B**) alanine aminotransferase (ALT): 291 days (95% CI, 109.8 to 472.2); (**C**) alkaline phosphatase (ALP): 184 days (95% CI, 4.4 to 363.6); (**D**) γ-glutamyl transferase (GGT): 63 days (95% CI, 61.44 to 64.6); and (**E**) TB: 120 days (95% CI, 74.6 to 164.5).

Other studies have found abnormalities in the levels of at least some liver enzymes [6, 8, 9, 14, 15]. We found a TB level of 14.3 ± 3.2 mg/dL (range: 6.3–22.4 mg/dL), which is comparable to the levels reported by Uras et al. [15] and Gartner et al. [16], but higher than the levels reported by Tazawa et al. [14] and Siu et al. [9]. The mechanism for hyperbilirubinemia in BMJ is not completely clear. Many hypotheses have been postulated, including inhibition of hepatic glucuronyl transferase (an enzyme promoting the conjugation of bilirubin) [3, 7, 14, 17], prominent increase of free fatty acid concentration

Table 3. Total bilirubin level for each group at the first presentation and at age 2 months

Group	Total bilirubin level (mg/dL) mean ± standard deviation			
	First presentation	*P*	Age 2 months§	*P*
Normal liver enzymes	14.1 ± 4.1	0.76	2.2 ± 1.7	0.09
Elevated liver enzymes	14.4 ± 2.7		4.1 ± 3.5	

§excluding subjects exposed to phototherapy (n = 1) and phenobarbital (n = 4).

(which inhibits conjugation) [3, 18], and increase of intestinal bilirubin absorption with a resultant increase in enterohepatic circulation [3, 18]. In the present study, the level of GGT (211.5 ± 70.3 U/L; range: 106–352 U/L) was the most frequently abnormal (in 19 of 42 infants; 45%), and this level and frequency are higher than those reported by Tazawa et al. (90–203 U/L; 13.8%, respectively) [14]. An explanation for the increased GGT levels found in BMJ is the extremely high GGT activity of breast milk during the first weeks of lactation; therefore, GGT in breastfed infants may be increased by absorption of GGT from breast milk [1]. ALT was the second most frequently elevated enzyme level (at 26%), which was higher than the enzyme level (8.4%) reported by Siu et al. [9]. ALT was not always abnormal at the first presentation but became elevated at the age of 2 months in around 75% of infants. This finding is consistent with the case report by Poddighe et al. [1], who reported that ALT was elevated when the infant was 4 weeks old. In the present study, the level of ALT was 37.5 ± 22.1 U/L (range: 10–133 U/L), and the highest level found was lower than the level (165 U/L) reported by Poddighe et al. [1], but the median level was higher than the level (21.8 U/L) reported by Siu et al. [9]. AST and ALP were the third most frequently elevated enzymes (21% each). The prevalence and the highest level of AST were lower than those reported previously [1, 8]. In breastfed infants, a higher serum level of AST is believed to result from higher liver metabolism, rather than hepatocyte damage [9]. The prevalence of an elevated level of ALP was comparable to that reported previously, but the highest level (913 U/L) was more than double the level (60 King–Armstrong units, or about 426 U/L) reported by Tazawa et al. [14].

The primary objective of the present study was to determine the time it takes for the abnormal liver enzyme levels to normalize. Here, we report the median time because the data were not normally distributed. The duration was estimated in the group with elevated liver enzyme levels because the infants in the group with normal liver enzyme levels were monitored only until they were 2 months old. The TB levels at the first presentation and at the age of 2 months were not significantly different between the 2 groups. We found that the median time for elevated serum levels of TB to normalize was 120 days, consistent with the findings by Jørgensen et al. [6] that TB was still high in breastfed infants when they were 6 months old. By contrast, other studies found that TB levels became normal within 3 months [2, 9]. From a clinical perspective, regardless of how long the serum level of TB remains elevated, interrupting breastfeeding and supplementing feeding with formulas based on cow's milk is not recommended [5]. We found that GGT had the most prevalently elevated levels, although the median time for the level to normalize was the shortest at 63 (range: 10–190) days, which is comparable to the finding that the GGT levels decline rapidly to adult levels by the time the infant is 6 months old [1, 19]. To our knowledge, this is the first study to follow infants with BMJ until the elevated serum levels of all measured liver enzymes declined to normal. For this reason, there are no available data with which to compare the duration of elevated liver enzyme levels, except for a single case report from Italy, which found that an infant was aged 6–7 months before ALT and AST levels normalized [1].

Ultimately, all infants in the group with elevated liver enzyme levels who completed the study (n = 13; or 48%) had normal GGT, ALT, ALP, and AST levels. All infants were otherwise healthy and developed normally during follow-up.

Limitations of this study include the substantial proportion of infants (n = 17; or 40%) who were lost to follow-up and who were excluded from the study because of formula feeding (10%). It should also be noted that although the number of enrolled infants was not large, the results are valid and generalizable and thus warrant extension of the study to a larger multicenter cohort.

Conclusions

The prevalence of elevated serum levels of liver enzymes in Thai infants with BMJ was higher than that expected from the literature. However, the levels gradually became normal within 1 year without any treatment, even though all infants remaining in the study continued to be exclusively breastfed. Pediatricians and general practitioners who may encounter infants with BMJ and transiently elevated liver enzyme levels may use a "watchful waiting" strategy in management of asymptomatic patients instead of performing sophisticated and unnecessary investigations.

Author contributions. All authors contributed to the conception and design of the study. PK acquired the data and

analyzed it. All authors contributed substantially to drafting and critically revising the manuscript, approved the final version submitted for publication, and take responsibility for the statements made in the published article.

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Conflict of interest statement. The authors have each completed and submitted an International Committee of Medical Journal Editors Uniform Disclosure Form for Potential Conflicts of Interest. None of the authors has any potential conflict of interest to disclose.

Data sharing statement. Data generated or analyzed during the present study are included in this published article. Data that support the findings of this study are also available in figshare, with the identifier https://doi.org/10.6084/m9.figshare.12234602; and all raw data are available from the corresponding author on reasonable request.

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