

Original Article

High hepatitis B seroprevalence and risk factors for infection in pregnant women on the Thailand-Myanmar Border

Tristan Banks¹, Joy Khang¹, Isabella Watts¹, Mary Ellen G Tyrosvoutis¹, Aung Myat Min¹, Nay Win Tun¹, Lily Keereecharoen¹, Wiriya Simmawong¹, Sunaree Wanyatip¹, Borimas Hanboonkunupakarn², François Nosten^{1,3}, Rose McGready^{1,3}

¹ Shoklo Malaria Research Unit, Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Mae Sot, Thailand

² Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

³ Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom

Abstract

Introduction: Infection from Hepatitis B primarily results from peri-partum vertical transmission and the risk increases in the presence of hepatitis B e antigen. We aimed to evaluate a new screening program for hepatitis B in pregnant women as a component of antenatal services in a marginalized population.

Methodology: Counseling and screening for hepatitis B screening was offered to all women at the first visit, at Shoklo Malaria Research Unit (SMRU) antenatal clinics on the Thai-Myanmar border. Point-of-care rapid diagnostic tests (RDT) were used throughout the period of evaluation. A certified Thai Public Health laboratory at Mae Sot Hospital verified RDT positive cases using enzyme-linked immunosorbent assay (ELISA) for HBsAb and HBeAg. Risk factors for hepatitis B were identified by data linkage to antenatal care records.

Results: There were 523 (8.5%) RDT positive for HBsAg among 6158 women tested (Aug-2012 to April-2014). Of these 373 (96.9%) of 385 sent for confirmation were positive by ELISA i.e. RDT false positive rate of 3.1% (95% CI 1.7- 5.4). The overall confirmed HbsAg prevalence was 8.3% (511/6158) (95% CI 7.6-9.0). HBeAg prevalence was 32.7% (114/350) (95% CI 27.9-37.7) of cases tested. Risk factors for HBsAg positivity included age > 25 years (OR 1.24, CI 1.03-1.49, p 0.021) and Karen heritage (OR 1.73, CI 1.39-2.15, p < 0.01).

Conclusions: High hepatitis B seroprevalence amongst migrants and refugees accessing SMRU antenatal services likely reflects that of Kayin State, Myanmar, and perinatal prevention programs are required. False positive cases with HBsAg RDT complicate what is theoretically a straightforward screening.

Key words: hepatitis B; marginalized populations; perinatal transmission; prevention; limited resource setting.

J Infect Dev Ctries 2016; 10(4):384-388. doi:10.3855/jidc.7742

(Received 14 July 2015 – Accepted 31 August 2015)

Copyright © 2016 Banks *et al.* This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Introduction

An estimated 2 billion people are infected with the hepatitis B virus. Prevalence varies considerably throughout the world and is highest in sub-Saharan Africa and Asia. It is the 10th leading cause of death worldwide, with mortality attributable to long-term sequelae, namely liver cirrhosis and hepatocellular carcinoma [1]. South-East Asia is considered an intermediate to high hepatitis B endemic area, with prevalence of up to 10% [2,3]. Although the prevalence in South-East Asia is decreasing [3], pockets of high endemicity remain in high risk groups and migrants from areas with high prevalence [4].

Perinatal transmission of the hepatitis B virus accounts for up to 50% of disease spread. It is well

documented that the earlier in life a person is exposed to the hepatitis B virus, the more chance there is of progression to chronic hepatitis B infection and long term consequences [3]. The mechanisms of transmission from mother to child include placental leakage and exposure to blood and secretions during delivery. The risk of transmission is greater in the presence of hepatitis B e antigen (HBeAg) or high viral loads. Infection rates are as high as 85-90% when HBeAg is present, compared to 5-31% when only hepatitis B surface antigen (HBsAg) is present [5].

The risk of perinatal transmission can be significantly reduced with hepatitis B vaccination and immunoglobulin administered soon after birth. The use of hepatitis B vaccination alone in the neonatal period

has been shown to be effective and hepatitis B immunoglobulin used in conjunction with hepatitis B vaccine decreases transmission further [6]. Hepatitis B control programmes, including vaccination of newborns, could reduce deaths related to hepatitis B infection by over 80% [7]. Despite development of vaccination programmes and overall reductions in prevalence, infections rates remain high in populations with poor access to healthcare [3,8].

This descriptive research aimed to evaluate a new screening programme for hepatitis B as a component of antenatal services to determine seroprevalence and risk factors amongst the pregnant women population, consisting primarily of refugees and migrants mostly from Kayin state, Myanmar, with a view to programme improvement.

Methodology

Setting

Shoklo Malaria Research Unit (SMRU) has jointly conducted research and provided humanitarian health care for refugee and migrant populations on the north western border of Thailand, neighboring Kayin State, Myanmar. Free antenatal care and birthing services can currently be obtained at three sites: two migrant sites including Wang-Pha (WPA) and Mawker Thai (MKT) and one refugee site known as Maela (MLA) camp. Health care vulnerability amongst refugee and migrant populations remains in a protracted state in this area. Refugee camps were established in 1984 and SMRU started to offer free antenatal and delivery care in 1986. In migrants the antenatal care services commenced in late 1998. Health care issues amongst migrant workers in Thailand and ASEAN are recognized [9] and in Tak Province with a large presence of registered and unregistered migrants, described for certain infectious diseases such as malaria [10] and tuberculosis [11] and pertaining to reproductive health care [12].

Hepatitis B was recognized locally as a problem from cross-sectional surveys conducted in February 1998 by SMRU in MLA using population census data. In this survey that controlled for age, sex and section of the camp a venous blood sample was tested for HBsAg using Hepatitis B STAT-TEST (Immuno-Chemical Lab Co. Ltd, France) a kit based on direct monoclonal chromatographic assay. In 387 samples 14.5% (56) were HBsAg positive with 16.7% (17/106) (95%CI 10.2-24.3) in females of 15 years and over. Following this survey the main health provider at the time, Médecins Sans Frontières, pledged to introduce newborn Hepatitis B vaccination for the refugee

population and this was established 1999 and has been continued ever since.

Participants

All women attending antenatal clinics from August 2012 and April 2014 were offered screening for hepatitis B after counseling about the disease. The hepatitis B test was offered at the same time as the HIV test which has a low prevalence and high test uptake in this setting as described previously [13]. Attendance at antenatal care is voluntary and uptake is thought to be high as the two organizations providing maternal and child health rarely identify infants born to mothers who have not attended SMRU antenatal care. In the migrant population attendance and delivery has continued to increase in part because access in rural areas to maternal and child health services provided in Karen and Burmese language are limited.

Record linkage

Demographic information was extracted from the antenatal care records, including ethnic group, number of marriages, history of receiving a blood transfusion at any time, and length of residence at current address. This data was matched to laboratory data of hepatitis B status.

Laboratory methods

Initial screening was performed using the *One Step Bioline Hepatitis B Surface Antigen Test Strip* rapid diagnostic test (Pacific Biotech, Thailand). At programme start up the point-of-care RDT was thought to be sufficient. However, at one of the sites during routine screening for blood donation of a SMRU staff member (who often respond to calls for urgent blood donation where no blood bank is available) complained that her status had changed from negative to positive. Confirmation at the local hospital in Thailand determined this to be a false positive RDT result. From this point all RDT HBsAg-positive cases for pregnant women were verified using the *HbSAg electrochemiluminescence immunoassay* (ECLIA) on Cobas e immunoassay analyzer ((Roche Diagnostics, Indianapolis, USA) conducted independently at the local tertiary referral provincial hospital in Thailand. This assay has been CE marked according to Directive 98/79/EC. The same laboratory carried out *HBeAg electrochemiluminescence immunoassay* (ECLIA) on Cobas e immunoassay analyzer ((Roche Diagnostics, USA) to confirm the presence or absence of carriage.

Statistical analysis

Data were analysed using STATA version 11. Demographic information was compared using the Chi-squared test for categorical variables and the Student's *t*-test or Mann-Whitney-U test for continuous data.

Potential risk factors for HBsAg positivity were analysed using univariate logistic regression. Multivariate logistic regression was performed for significant or near-significant variables (*p*-value < 0.1) identified by univariate logistic regression. The variable parity was excluded from the multivariate logistic regression model because of collinearity with age.

Ethics

Retrospective review of anonymized data from antenatal records was approved by the local Tak Community Advisory Board and the Oxford Tropical Research Ethics Committee (OXTREC 28-09).

Results

A total of 6176 pregnancies were seen by SMRU staff during the period described. A result for HBsAg was not available in 18 cases and these were excluded from further analysis. Reasons for failure of screening included women presenting late in their pregnancy with complications requiring urgent transfer, women leaving directly after antenatal ultrasound before blood could be taken, and women presenting with miscarriage going straight to the delivery room. Demographic information for women at each site, was summarized (Table 1).

The 114 women who attended for more than one pregnancy during the screening period did not change HBsAg status as tested by RDT. These women were only included for their initial pregnancy. HBsAg rapid diagnostic tests were positive in 523 (8.5%) of the 6158 included cases.

Prevalence of HBsAg and HBeAg

HBsAg RDT results were sent for confirmation for 385 (76.1%) positive cases. ELISA confirmed HBsAg positivity in 373 (96.9%) but provided a negative result in 12 (3.1%). Hence, the RDT false positive rate in this setting was 3.1% (95% CI 1.7- 5.4).

HBeAg testing was requested in 350 (97%) of the 385 HBsAg RDT positive cases and was positive in 114 (32.6%) of these. Missed requests were due to a misunderstanding at one site on the tests to be checked on the request form for the public hospital.

To determine the overall prevalence the 12 false positive results were counted as negative giving a total HbsAg positive count of 511 in 6158 women and a prevalence of 8.3% (95%CI 7.6-9.0%). The HBeAg prevalence was 32.7% (95%CI 27.9-37.7%).

Factors associated with hepatitis B in pregnant women

Factors identified by univariate logistic regression to be associated with HBsAg RDT positivity were maternal age of over 25 years (OR 1.22, CI 1.02-1.47, *p* 0.032), having refugee rather than migrant status (OR 1.32, CI 1.10-1.57, *p* < 0.01), multigravidity (OR 1.29,

Table 1. Demographic information of women attending antenatal care at each site.

Characteristics	Maela		Maw Ker Thai		Wang Pha		p1	p2	p3
	N=2483		N=1695		N=1980				
Age in years	26±7	14-50	26±7	15-48	27±7	14-47	0.401	0.027	0.005
Gravidity	2	1-15	2	1-19	2	1-12	0.503	0.67	0.304
Primigravidae	32.1	798	33.5	567	30.9	611	0.374	0.361	0.093
Number of marriages per woman	1	1-4	1	1-5	1	1-5	<0.001	<0.001	0.844
Myanmar address	10.4	259	31.4	532	67.7	1340	<0.001	<0.001	<0.001
Years at current address	7	0-44	3	0-45	2	0-46	<0.001	<0.001	0.275
Literate	62.1	1543	63.4	1075	51.7	1023	0.401	<0.001	<0.001
<i>Ethnic group</i>									
Sgaw Karen	72	1789	27.8	471	30.2	597	<0.001	<0.001	0.116
Mixed Karen (Sgaw and Poe)	2.6	65	3.2	54	3.1	61	0.278	0.354	0.855
Poe Karen	8	199	12.5	211	18.3	362	<0.001	<0.001	<0.001
Burmese Muslims	11.6	289	0.4	6	0.3	5	<0.001	<0.001	0.575
Burman	2	51	43	729	40.2	796	<0.001	<0.001	0.085
Mixed Karen and Burmese	2	50	5.4	92	3.9	78	<0.001	<0.001	0.032
Muslim/Burman/Other									
Other ^b Ethnic group e.g. Mon, Pa-Oh, Rakhine, Shan, Chin , other mixed ethnic group	1.5	37	7.4	126	3.9	78	<0.001	<0.001	<0.001
Burman and Burmese Muslims	0.1	3	0.4	6	0.2	3	0.11	0.781	0.216

Dichotomous data is represented as %(number). Continuous data as mean±SD (range) for parametric and median (range) for non-parametric; p1 = MLA vs. MKT, p2 = MLA vs. WPA, p3 = WPA vs. MKT. *missing for 3 cases

CI 1.06-1.58, p 0.012) and being of Karen ethnicity (OR 1.79, CI 1.45-2.19, p <0.01). Literacy (OR 0.88, CI 0.73-1.05, p 0.157), history of blood transfusion (OR 1.22, CI 0.84-1.78, p 0.298) and previous marriages (OR 0.93, CI 0.73-1.19, p 0.569) were not significantly associated with HBsAg positivity. In multivariate analysis, maternal age of over 25 years (OR 1.24, CI 1.03-1.49, p 0.021) and Karen ethnicity (OR 1.73, CI 1.39-2.15, p <0.01) were significant.

HBeAg carriage was associated with lower maternal age. The proportion of HBeAg positivity in Karen (88/254, 34.7%) was higher than in non-Karen (26/93, 28%) ethnic groups but was not significant (P =0.240).

Discussion

HBsAg prevalence

We have identified a high hepatitis B surface antigen seropositivity among pregnant women on the Thai-Myanmar border. The overall HBsAg prevalence in this population is higher than recent estimates for South-East Asia as a whole [3] but similar to estimates of nearby regions [4,14]. It has previously been shown that the prevalence of hepatitis B infection is higher in men than in women [3,4,14]. Therefore, it is likely that the overall prevalence in the population of migrants and refugees on the Thai-Myanmar border is even higher. These findings are consistent with previous reports of high HBsAg positivity in migrant workers from Myanmar [8]. Although not directly comparable the rate current rate in pregnant women of 8.3% (95%CI 7.6-9.0%) is lower than observed in females aged 15 and over in a former cross-sectional survey conducted in Maella camp in 1998: 16.7% (95%CI 10.2-24.3). This may reflect some success of the newborn vaccination programme which commenced in 1999 and also the lower rate of HBsAg positive in those less than 25 years of age in the current survey. The camp population is not stable making an evaluation of the programme difficult without actually testing for hepatitis B infection in young infants.

In the 375 RDT positive cases that were verified using ELISA, we found an RDT false positive rate of 3.1%. Studies estimating the sensitivity and specificity of the Bioline HBsAg RDT have reported specificity of 100% and sensitivity of 97.95% and higher [15,16]. Factors affecting the accuracy of rapid diagnostic tests for HBsAg include the geographical region of the sample source and the concentration of HBsAg in the sample [17]. We are unable to comment on the sensitivity and specificity of this test in our population because of the limitations of only confirming the RDT

positive cases. In order to screen potentially infectious blood donors false positive results are acceptable but in order to counsel a person that they a potentially life long infection and risk transmitting the infection to the infant, this rate of false positives is unacceptable. In addition, it was detected incidentally: staff, former blood donors were asked to donate blood in an emergency and were upset when they learned they were HBsAg positive when they were previously negative, a requirement to be a blood donor. Confirmation of their status by the local provincial hospital confirmed the false positive rapid diagnostic test and this serves as a reminder of the limitations of the RDT.

HBeAg prevalence

The proportion of HBsAg positive women who also tested positive for HBeAg 32.7% (95%CI 27.9-37.7%) is comparable to previous estimates in South-East Asia [3]. These represent pregnancies with the highest risk of perinatal transmission and should be the target of interventions aimed at prevention of transmission. This programme evaluation has led to the implementation of newborn hepatitis B immunoglobulin for HBeAg positive cases.

Risk factors for HBsAg seropositivity

We found Karen ethnicity to be an independent predictor of HBsAg positivity. Variations in prevalence by ethnic group have been demonstrated in similar populations [14]. This finding is important in order to prioritize public health interventions to target the most at-risk groups. Further population-based studies of hepatitis B prevalence in Myanmar are needed. This high prevalence in this Karen population is probably explained by the absence of immunization programme in the refugee camps and in their places of origin (Myanmar).

Other potential risk factors assessed, including the receipt of blood transfusion and having had more than one marriage (a surrogate for number of sexual partners), were not found to be associated with HBsAg positivity. This suggests that the majority of transmission in this population is vertical. A limitation of this study is lack of information regarding the use of intravenous drugs. We suspect intravenous drug use to be low in this population, where intravenous drug abuse is culturally unacceptable and heavily punished [13,18].

Conclusions

This is the first study to assess the prevalence of hepatitis B in the pregnant population on the Thai-Myanmar border. We have found a high prevalence of

hepatitis B seroprevalence and significant risk factors include having either parent of Karen ethnicity, having refugee rather than migrant status, and having had one or more previous pregnancies. Despite introduction of the vaccination programme 15 years prior a high proportion of pregnant women are chronic hepatitis B carriers and efforts to reduce perinatal transmission with, for example, anti-retrovirals during pregnancy should be considered marginalized populations where population movement and remoteness may have limited the effectiveness of vaccination programmes.

Acknowledgements

We are grateful to the pregnant women who attend antenatal care and hope we can continue to realize improvements in health problems locally and internationally. The work would not be possible without the continued support of the midwives, laboratory and logistic staff who still smile when we ask if the sample box is ready.

References

1. Lavanchy D (2004) Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. *J Viral Hepat* 11: 97-107.
2. Custer B, Sullivan SD, Hazlet TK, Iloeje U, Veenstra DL, Kowdley KV (2004) Global epidemiology of hepatitis B virus. *J Clin Gastroenterol* 38 Suppl 3: 158-168.
3. Ott JJ, Stevens GA, Groeger J, Wiersma ST (2012) Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBsAg seroprevalence and endemicity. *Vaccine* 30: 2212-2219.
4. Heathcote EJ (2008) Demography and presentation of chronic hepatitis B virus infection. *Am J Med* 121 Suppl 12: 3-11.
5. Ott JJ, Stevens GA, Groeger J, Wiersma ST (2012) The risk of perinatal hepatitis B virus transmission: hepatitis B e antigen (HBeAg) prevalence estimates for all world regions. *BMC Inf Dis* 12: 131.
6. Lee C, Gong Y, Brok J, Boxall EH, Gluud C (2006) Hepatitis B immunisation for newborn infants of hepatitis B surface antigen-positive mothers. *The Cochrane Database Syst Rev*: CD004790.
7. Goldstein ST, Zhou F, Hadler SC, Bell BP, Mast EE, Margolis HS (2005) A mathematical model to estimate global hepatitis B disease burden and vaccination impact. *Int J Epidemiol* 34: 1329-1339.
8. Sa-Nguanmoo P, Tangkijvanich P, Thawornsuk N, Vichaiwattana P, Priantathavorn K, Theamboonlers A, Tanaka Y, Poovorawan Y (2010) Molecular epidemiological study of hepatitis B virus among migrant workers from Cambodia, Laos, and Myanmar to Thailand. *J Med Virol* 82: 1341-1349.
9. Guinto RL, Curran UZ, Suphanchaimat R, Pocock NS (2015) Universal health coverage in 'One ASEAN': are migrants included? *Glob Health Action* 8: 25749.
10. Carrara VI, Lwin KM, Phyo AP, Ashley E, Wiladphaingern J, Sriprawat K, Rijken M, Boel M, McGready R, Proux S, Chu C, Singhasivanon P, White N, Nosten F (2013) Malaria burden and artemisinin resistance in the mobile and migrant population on the Thai-Myanmar border, 1999-2011: an observational study. *PLoS Med* 10: e1001398.
11. Minetti A, Camelique O, Hsa Thaw K, Thi S, Swaddiwudhipong W, Hewison C, Pinoges L, Bonnet M, Guerin PJ (2010) Tuberculosis treatment in a refugee and migrant population: 20 years of experience on the Thai-Burmese border. *Int J Tuberc Lung* 14: 1589-1595.
12. Sullivan TM, Sophia N, Maung C (2004) Using evidence to improve reproductive health quality along the Thailand-Burma border. *Disasters* 28: 255-268.
13. Plewes K, Lee T, Kajeewchewa L, Thwin MM, Lee SJ, Carrara VI, Nosten F, McGready R (2008) Low seroprevalence of HIV and syphilis in pregnant women in refugee camps on the Thai-Burma border. *Int J STD AIDS* 19: 833-837.
14. Ishida T, Takao S, Settheetham-Ishida W, Tiwawech D (2002) Prevalence of hepatitis B and C virus infection in rural ethnic populations of Northern Thailand. *J Clin Virol* 24: 31-35.
15. Maity S, Nandi S, Biswas S, Sadhukhan SK, Saha MK (2012) Performance and diagnostic usefulness of commercially available enzyme linked immunosorbent assay and rapid kits for detection of HIV, HBV and HCV in India. *Virol J* 9: 290.
16. Scheiblaue H, El-Nageh M, Diaz S, Nick S, Zeichhardt H, Grunert HP, Prince A (2010) Performance evaluation of 70 hepatitis B virus (HBV) surface antigen (HBsAg) assays from around the world by a geographically diverse panel with an array of HBV genotypes and HBsAg subtypes. *Vox Sang* 98: 403-414.
17. World Health Organization (2004) Hepatitis B surface antigen assays: operational characteristics (Phase 1) Report 2. Available: <http://apps.who.int/medicinedocs/en/d/Js15206e/> Accessed 01 May 2015.
18. Ezard N, Thiptharakun S, Nosten F, Rhodes T, McGready R (2012) Risky alcohol use among reproductive-age men, not women, in Mae La refugee camp, Thailand, 2009. *Confl Health* 6: 7.

Corresponding author

Rose McGready (MD, PhD)
Department of Maternal and Child Health
Shoklo Malaria Research Unit, 68/30 Baan Tung Road, 63110,
Mae Sot, Thailand
Phone: +66 55 545 021
Fax: +66 55 545 020
Email: rose@shoklo-unit.com

Conflict of interests: No conflict of interests is declared.