

Original Article

નિયન્ત્રિત નાણબંન

Maternally Transferred Antibody of Dengue and Chikungunya Viruses in Pregnant Thai Women and Their Infants

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Abstract

In a cross-sectional study at Ban Pong Hospital, Ratchaburi Province, Thailand, 147 maternal-cord paired sera were tested for dengue and chikungunya antibodies by hemagglutination inhibition assay (HAI). Nearly 94 percent of pregnant women had HAI antibody to at least one dengue serotype; the proportion of antibody to DEN-1 was 93.2, DEN-2 92.5, DEN-3 93.8, and DEN-4 93.2 percent. Almost all of the infants had the same proportion of seropositivity to each dengue serotype. Antibody titer was highest in DEN-3, followed by DEN-4 and DEN-1, in both maternal and cord sera. The lowest titer was shown for DEN-2 antibody. The seroprevalence of chikungunya antibody in maternal-cord sera was much lower, at 8.2 percent, with a low HAI titer of 6.1. In this dengue-endemic area, most infants had maternally transferred dengue antibody, which may impact upon disease burden among this population.

Key words: dengue, chikungunya, pregnant women, infants, transplacental transferred antibody

Introduction

The World Health Organization (WHO) has declared dengue the fastest-spreading mosquito-borne viral disease in the world.⁽¹⁾ Infection with any one of the 4 antigenically-related serotypes - DEN-1, DEN-2, DEN-3, or DEN-4 - can produce a broad spectrum of effects, including asymptomatic infection, undif-

ferentiated febrile illness, dengue and severe dengue infection.⁽²⁾ This potentially fatal disease infects up to 500 million people and causes more than 22,000 deaths each year, mainly among children and young adults.^(3,4) Epidemic dengue occurs in Asia, the Americas, and some Pacific islands. The WHO regions of Southeast Asia (SEA) and the Western Pacific repre-

sent approximately 75 percent of the current global burden of dengue.^(2,5) In Asia, dengue mainly affects children, for whom it is among the 10 leading causes of hospitalization and death.⁽⁶⁾

The first Thai dengue patient was treated in Bangkok in 1958; dengue then began to appear in other parts of the country.^(4,7) Ratchaburi Province is located 100 kilometers from Bangkok, Thailand's capital, and in 2011 was reported as being among the top 10 provinces in terms of the incidence of dengue. From 2000 to 2010, the rate of dengue patients in Ratchaburi Province, Thailand, varied from 123.45 per 100,000 population in 2003, to 394.25 in 2008.⁽⁸⁾

Dengue is a mosquito-borne viral disease transmitted by the vectors *Aedes aegypti* and *Aedes albopictus*. The pathogenesis of severe dengue, including dengue hemorrhagic fever (DHF), is still not clearly understood. Observations recorded early on in Bangkok were that infants aged < 12 months with dengue viral infection were at higher risk of developing DHF if maternal antibodies to dengue virus were present at subneutralizing levels; this observation led to theories of DHF being caused by antibody-dependent enhancement (ADE) of viral infection.⁽⁹⁻¹²⁾ Antibodies, predominantly of the IgG subclass, are the only immunological substances known to be transferred from mother to fetus⁽¹³⁻¹⁵⁾. The high level of transferred neutralizing dengue antibodies in infants at delivery have been demonstrated in previous reports.^(16,17) Infants from SEA fail to develop clinical dengue until around 6-9 months of age due to the presence of transferred dengue neutralizing antibodies, which have been found in their serum samples.^(6,16-18)

An important strategy for dengue prevention and control is the development of an effective dengue vaccine. A multicenter phase III dengue vaccine trial on the Asian and Latin American continents has been ongoing since 2011; Ban Pong and Photharam districts in Ratchaburi Province, and Kamphaeng Phet

Province, Thailand, are included in the trial. Future vaccination strategies will depend on reliable data on the kinetics of maternal dengue antibodies, as well as on accurate measurements regarding disease impact.

Chikungunya infection, caused by the chikungunya virus, is similar to dengue in terms of its shared mosquito vectors and its signs and symptoms during the early stages of infection. Chikungunya fever was first detected in Bangkok, also in 1958; according to the records, it subsequently disappeared from Thailand for about 14 years, until 1976. The disease re-emerged from 1991 to 1995, and then again in mid-August 2008 in Narathiwat, the southernmost province of Thailand, from where it spread nationwide.⁽¹⁹⁻²¹⁾ According to the Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Thailand, the incidence of chikungunya infection was 3.95, 82.03, and 2.46 per 100,000 population in 2008, 2009, and 2010, respectively.⁽²¹⁾

The objectives of this study were to estimate the seroprevalence of dengue and chikungunya antibody among mothers living in an area of high endemicity - Ban Pong, Ratchaburi Province - and to assess maternal dengue and chikungunya antibodies transferred to infants.

METHODS

Ban Pong Hospital is a 300-bed government-run hospital, serving a Ban Pong District population of about 169,900 and delivering an average 160 newborns per month. In this cross-sectional study, 151 mother-infant pairs were enrolled at Ban Pong Hospital from December 2011 to January 2013. The principal exclusion criteria for mothers were: maternal history of immune deficiency, receiving immunosuppressive treatment within the previous month (including systemic corticosteroid therapy for > 2 weeks), and receiving blood or blood components within the previous 3 months.

The sample size required to determine the prevalence of dengue infection among pregnant women was calculated using Epi Info software (2002). Based on the survey among pregnant women in Bangkok during 2000-2001, the prevalence for dengue antibody was 97 percent, with a confidence level of 95 percent, and precision of +/- 3 percent, the sample size was 124. Thus, the total sample size for this study was

Table 1 Demographic characteristics of 151 pregnant women and infants.

Variable	Number (%)
Age (years)	
≤20	55 (37.4)
21-25	42 (28.6)
26-30	23 (15.7)
31-35	24 (16.3)
≥36	3 (2.0)
Parity	
0	66 (43.7)
1	55 (36.4)
>2	30 (19.9)
Education	
primary school	59 (39.1)
secondary school	73 (48.3)
vocational school	11 (7.3)
bachelor degree	8 (5.3)
Occupation	
no	71 (47.0)
employee	59 (39.1)
others	21 (13.9)
Monthly household income (THB)	
<10,000	115 (76.2)
≥10,000	36 (23.8)
Gestational age (wks)	
<37	1 (0.7)
37-41	149 (98.6)
≥42	1 (0.7)
Mode of delivery	
Spontaneous delivery	143 (94.7)
Vacuum extraction	2 (1.3)
Cesarean section	6 (4.0)

adjusted up to 150 pregnant women. The study protocol was approved by the Ethics Committee of Ban Pong Hospital. Written informed consent was provided by all mothers before enrollment.

The demographic characteristics of the mothers and newborn were documented within 24 hours of birth. At delivery, 5-ml blood samples were collected from the mothers, as well as umbilical cords for dengue and chikungunya serological study.

Serology. All samples were centrifuged within a few hours after collection and stored at -20°C. Assays were performed at the laboratory of the Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand. Antibody levels against all 4 dengue types, as well as Japanese encephalitis (JE) and chikungunya reference strains, were determined. Hemagglutination inhibition (HAI) serological analysis was performed according to the method of Clarke and Casals, as modified for the microtiter system, as previously described.⁽²²⁾ HAI titer $\geq 1:10$ to one dengue serotype at least was considered seropositive, while titer $< 1:10$ to each dengue serotype was seronegative. The same criteria were applied for chikungunya antibody.

Data analysis. Serotype-specific seroprevalence rates were calculated as the proportion of samples with HAI levels $\geq 1:10$ against at least one dengue serotype. Geometric mean titer (GMT) and 95 percent confidence interval (CI) were calculated for each serotype. The seroprevalence of chikungunya infection in the mothers was calculated using the same criteria. The level of significance was set at 0.05 p-value for comparisons of seroprevalence based on symmetry test; p-value for comparison of GMT between that of mother and cord was based on paired test of log (HAI), Spearman correlation < 0.001 was used for correlation between maternal and cord blood of DEN-1 to DEN-4 antibody titers. All statistical analyses were performed using STATA software, version 10.

Table 2 The proportion and geometric mean titer of dengue and chikungunya antibodies of 147 maternal and cord paired sera.

	Positive (n)	%	GMT	(95%CI)
DEN-1				
mother	137	93.2	77.77	62-11-97.37
cord	134	91.2	78.50	62.04-99.34
p-value		0.08		0.81
DEN-2				
mother	136	92.5	48.53	39.93-58.98
cord	133	90.5	47.85	39.02-58.68
p-value		0.08		0.69
DEN-3				
mother	138	93.9	95.04	76.44-118.15
cord	136	92.5	95.48	76.16-119.71
p-value		0.16		0.90
DEN-4				
mother	137	93.2	91.72	73.29-114.78
cord	136	92.5	90.01	71.11-113.92
p-value		0.56		0.59
CHIK				
mother	12	8.2	6.12	5.41-6.93
cord	12	8.2	6.09	5.40-6.87
p-value		1.00		0.89

Note: p-value for comparisons of seroprevalence is based on symmetry test; p-value for comparison of GMT between mother and cord is based on paired test of log (HAI)

Results

One hundred and fifty-one (151) mother-infant pairs were initially recruited. Mean (SD) maternal age was 23.5 years (5.7 years; range 15-41 years). All lived in Ban Pong, Ratchaburi Province. Maternal and cord sera at delivery were obtained for 147 pairs.

About one-third of the pregnant women were aged <20 years. Nearly 44 percent were primigravida. Demographic characteristics of the mothers - age, level of education, occupation, household income - and important profiles of their newborn infants, are shown in Table 1. At birth, all infants were healthy. Only two infants weighed < 2,500 grams (2,190 and 2,460 grams), while 98.6 percent of infants were full term. Mean birthweight (\pm SD) was 3,196 (\pm 399) grams,

ranged 2,190-4,430 grams. The male to female ratio was 1.1:1.

Most mothers (138/147, 93.9%) had HAI antibody to at least 1 dengue serotype (titer (10 1/dilution) in their serum. Proportions of seroprevalence of all 4 dengue serotypes and chikungunya virus, and GMT levels, among the pregnant women and their infants are shown in Table 2. At birth, the proportion of infants with HAI antibodies to the serotypes were high and similar to their mothers, at 91.2 percent for DEN-1, 90.5 percent for DEN-2, 92.5 percent for DEN-3, and 92.5 percent for DEN-4. The GMT levels of dengue HAI titers in cord blood were almost the same as the maternal antibodies: highest in DEN-3, followed by DEN-4, then DEN-1. While the GMT

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Table 3 Age -specific seroprevalence of maternal antibody to dengue and chikungunya viruses in mothers and cord blood.

		≤ 20 years n=55	21-25 years n=42	26-30 years n=23	31-35 years n=24	≥ 36 years n=3	p-value
DEN-1							
Mother	Positive	52	38	20	24	3	0.402
	(%)	94.6	90.5	86.9	100.00	100.00	
Cord	Positive	52	36	20	23	3	
	(%)	94.6	85.7	86.9	95.8	100.00	0.438
DEN-2							
Mother	Positive	51	38	20	24	3	0.483
	(%)	92.7	90.5	86.9	100.00	100.00	
Cord	Positive	50	36	20	24	3	
	(%)	90.9	85.7	86.9	100.00	100.00	0.368
DEN-3							
Mother	Positive	53	38	20	24	3	0.276
	(%)	96.4	90.5	86.9	100.00	100.00	
Cord	Positive	53	36	20	24	3	
	(%)	96.4	85.7	86.9	100.00	100.00	0.126
DEN-4							
Mother	Positive	52	38	20	24	3	0.402
	(%)	94.6	90.5	86.9	100.00	100.00	
Cord	Positive	53	36	20	24	3	
	(%)	96.4	85.7	86.9	100.00	100.00	0.126
CHIK							
Mother	Positive	0	1	5	5	1	<0.001
	(%)	0.00	2.4	21.7	20.8	33.3	
Cord	Positive	0	1	5	5	1	
	(%)	0.00	2.4	21.7	20.8	33.3	<0.001

Note: p-value is based on comparison of seroprevalence across 5 age groups

of DEN-2 was lowest among all the 4 serotypes, no significant differences were found. Maternal and cord HAI of DEN-1, DEN-2, DEN-3, and DEN-4 antibody titers correlated well (Spearman correlation < 0.001), as shown in Figure 1. In contrast to dengue infection, the seroprevalence of chikungunya infection was only 8.2 percent among the pregnant women, and all 12 infants obtained their maternal antibody with a low HAI titer of 6.1.

Table 3 shows the age-specific seroprevalence

of dengue and chikungunya infection in 5 age groups of the pregnant women. There was no statistically significant difference in dengue seroprevalence by maternal age grouping. No chikungunya antibody was demonstrated in mothers ≤ 20 years of age. However, 2.4, and percent seroprevalence was found in mothers aged 21-25 in 2.4, 26-30 in 21.7, 31-35 in 20.8 and ≥ 36 years old in 33.3 percent, which does represent a statistically significant difference ($p < 0.001$).

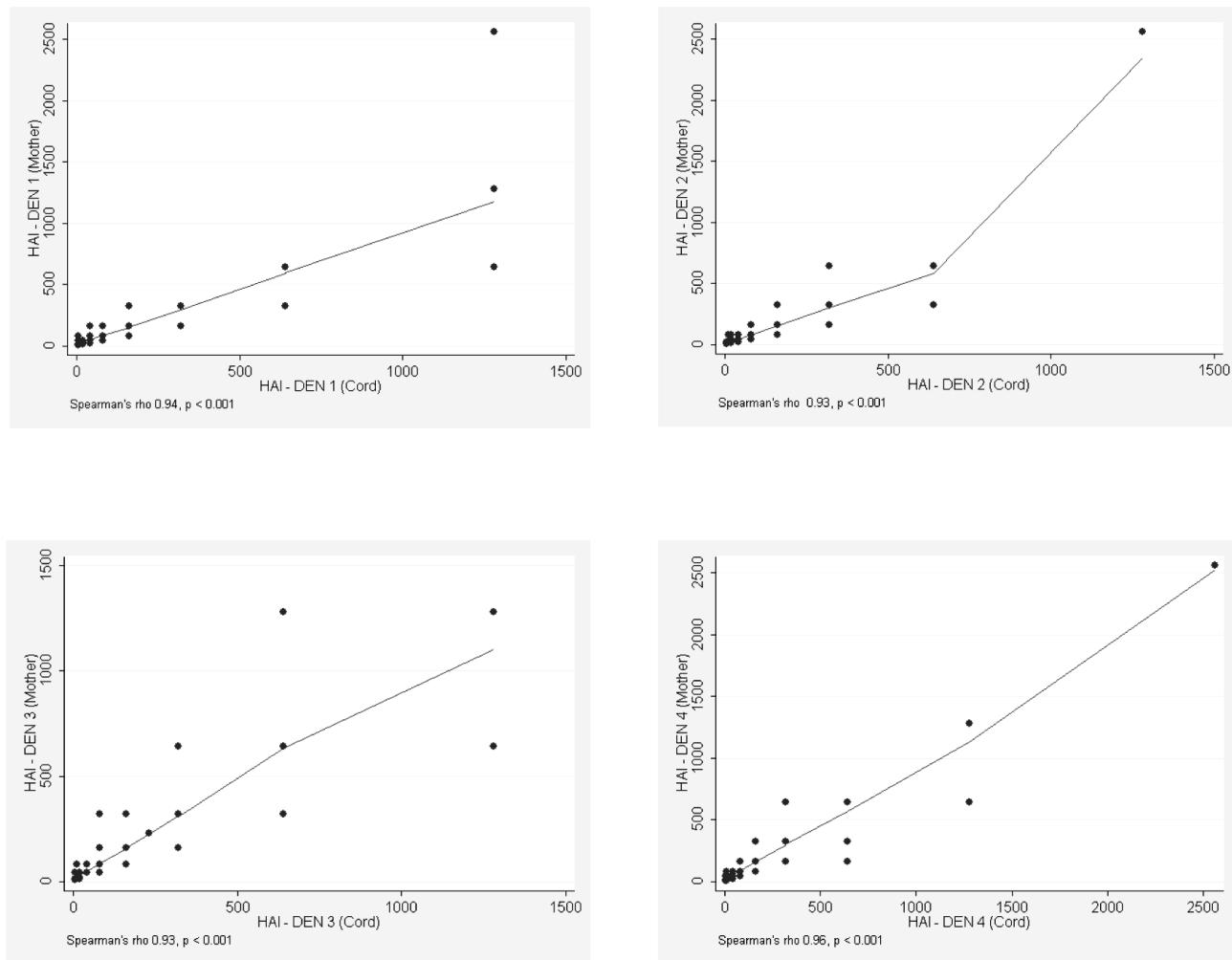


Figure 1. Correlation between maternal and cord blood of DEN-1 to DEN-4 antibody titers

Discussion

In this study, nearly 94 percent of the pregnant women who delivered their infants at Ban Pong Hospital displayed serological evidence of prior dengue infection. Most transferred dengue antibodies to their infants. The large proportion of mothers with antibodies against multiple dengue virus serotypes reflects the high rate of transmission of dengue viruses in Thailand. The high level of transferred antibodies at delivery in this study is consistent with previous findings in Bangkok, Thailand, during 1998-2001.^(16,17,23,24)

Most infants experienced a decline in maternal antibody level during the first 12 months, and these infants are at risk of developing various spectra of

dengue infection. Epidemiological, clinical, and virological studies indicate the disease severity could be due to viral virulence, as well as the presence of passively acquired, heterotypic sub-neutralizing maternal-transferred dengue antibodies causing ADE.⁽⁹⁻¹²⁾ A high rate of severe dengue in infants is associated almost exclusively with primary dengue infection.

Significant variations in both clinical features and in severity of dengue in children from different age groups have been reported, with central nervous system manifestations more common among infants.⁽²⁵⁻²⁷⁾ Undifferentiated fever and asymptomatic dengue infection were diagnosed in infants after a decline of maternal-transferred antibody.^(17,27) With this in mind,

refinement and reconsideration of the current ADE pathogenesis should be encouraged.⁽¹⁸⁾

Almost exclusively, all mothers aged > 30 years, together with their infants, showed a high prevalence of dengue antibody to all 4 dengue serotypes compared with mothers aged < 30 years. This is similar to a previous study in Bangkok, where all mothers aged > 35 years transferred antibodies to their infants.⁽²⁴⁾ The higher prevalence of dengue infection among older mothers compared to younger mothers reflects the high transmission rate of dengue viruses in Thailand, and confirms the findings of previous studies.^(17,23,24)

The prevalence of chikungunya infection in this study was much lower than the 33.6 percent reported in Bangkok for the year 2000.⁽²⁸⁾ The difference may be due to sample size, as well as to the actual time period of the study. Higher prevalence, however, was demonstrated in mothers aged 26-35 years. Histories of travel to endemic areas in southern Thailand, and the role this is playing, should be explored further.

The WHO's "Global Strategy for Dengue Prevention and Control, 2012-2020" addressed the growing need for the treatment of dengue, and targeted a reduction in morbidity of the disease by 25 percent and mortality by 50 percent (using 2010 estimates as a baseline) by 2020.^(1,29) Dengue prevention strategies employing both potential vaccines and sustainable vector control measures should be emphasized to achieve these goals. No licensed vaccine against dengue is currently available. An effective tetravalent dengue vaccine remains crucial for controlling the disease, especially among high-risk populations in dengue-endemic Southeast Asia and Latin America.

The protective efficacy of recombinant live-attenuated, CYD tetravalent dengue vaccine has been reported among Thai schoolchildren.⁽³⁰⁾ Up-to-date findings in highly endemic areas have important public-health implications, for example, in terms of the

appropriate age to administer dengue vaccine.

Conclusion

High dengue seroprevalence, and low chikungunya seroprevalence, were found among the pregnant women at Ban Pong Hospital. Almost all pregnant women in Ban Pong, Ratchaburi Province, have been infected with all 4 dengue serotypes. Most infants appear to be protected from dengue infection in early life by maternal-transferred dengue antibodies. All infants had the same level of seropositivity to each dengue serotype as their mothers. In this dengue-endemic area, maternally transferred dengue antibodies, and the duration of protection they afford, may impact disease burden among infants, and provide further insight into the optimal age for dengue vaccination.

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การถ่ายทอดภูมิคุ้มกันต่อไวรัสเดงกีและไวรัสชิคุนกุนยาจากมาตรการด้านสาธารณสุข

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มหาวิทยาลัย
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ผู้วิจัยได้เจาะเลือดหนูทึ้งครรภ์ในระยะคลอดบุตรและเก็บเลือดจากสายสะดื้อทารกเมื่อแรกคลอด 147 ราย และสั่งตรวจภูมิคุ้มกันต่อไวรัสเดงกีและไวรัสชิคุนกุนยาด้วยวิธี hemagglutination inhibition assay (HAI) พน. ว่าร้อยละ 94 ของหนูมีตั้งครรภ์ ในการศึกษาแบบภาคตัดขวางนี้มีภูมิคุ้มกันต่อไวรัสเดงกีอย่างน้อยหนึ่งสายพันธุ์โดยมีสัดส่วนของภูมิคุ้มกันต่อไวรัสเดงกีสายพันธุ์ที่ 1, 2, 3 และ 4 เท่ากับ ร้อยละ 93.2, 92.5, 93.8 และ 93.2 ตามลำดับ ทารกแรกเกิดที่มีภูมิคุ้มกันต่อไวรัสเดงกีสายพันธุ์ที่ 1, 2, 3 และ 4 ไปในสัดส่วนเดียวกันกับมาตรา ทั้งมารดาและทารกมีระดับภูมิคุ้มกันต่อไวรัสเดงกีสายพันธุ์ที่ 3 สูงสุดตามมาด้วยระดับภูมิคุ้มกันต่อไวรัสเดงกีสายพันธุ์ที่ 4, 1 และ 2 ตามลำดับ สำหรับภูมิคุ้มกันต่อไวรัสชิคุนกุนยานั้นพบเพียง ร้อยละ 8.2 โดยมีค่าภูมิคุ้มกันต่อไวรัสชิคุนกุนยาอยู่ในระดับต่ำซึ่งเท่ากับ HAI titer 6.1 โดยสรุปพบว่าทารกในการศึกษานี้ซึ่งอยู่ในแหล่งรังโกรกไว้เลือดออกมีภูมิคุ้มกันต่อไวรัสเดงกีที่ถ่ายทอดมาจากมาตรการดูแลอยู่ในระดับสูงซึ่งอาจส่งผลกระทบต่อการเกิดโรคไว้เลือดออกในทารกกลุ่มนี้

คำสำคัญ: ไวรัสไข้เลือดออก, ไวรัสชิคุนกุนยา, หนูตั้งครรภ์, ทารกแรกเกิด, ภูมิคุ้มกันจากการด้านสาธารณสุข