Seroepidemiology of Dengue Virus Infection in HIV-Infected Children in Comparison to Healthy Children

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Background: *Dengue infection is the most common arboviral infection in the world while the HIV/AIDS epidemic remains a global concern. The pathogenesis of both diseases is rather on the contrary and it is generally observed that dengue diseases are uncommon in children with AIDS.*
Objective: *To study the seroprevalence of dengue virus infection in HIV-infected children compared to healthy children.*
Material and Method: *A cross-sectional seroprevalence of dengue virus was conducted at King Chulalongkorn Memorial Hospital, Bangkok, Thailand. Eighty-six HIV-infected children aged less than 15 years and one hundred age-matched healthy children were enrolled. HIV-infected children were classified in categories by CDC 1994 criteria. Neutralizing antibodies to all four dengue serotypes (DEN1, DEN2, DEN3, and DEN4) were measured by plaque reduction neutralization test (PRNT).*
Results: *Fifty out of 86 (58%) HIV-infected children and 65 out of 100 (65%) healthy, HIV-negative children had positive neutralizing antibody against dengue virus by PRNT. There were no significant differences between these two groups ($p>0.05$). Most children had neutralizing antibody against DEN2. In HIV-infected children, a monotypic PRNT50 pattern was found in 26 children (30%) and multitypic pattern was found in 24 children (28%). Most children had neutralizing antibody against DEN2. There were no significant differences in dengue seroprevalence between these two groups.*
Conclusion: *HIV-infected children and healthy children had no different seroepidemiology of dengue virus infection.*

Keywords: *Dengue, HIV, Seroepidemiology, Children*

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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, undifferentiated febrile illness, dengue and severe dengue infection, a life threatening illness characterized by fever, thrombocytopenia, increased vascular permeability, and hemorrhagic diathesis[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. Using cartographic approaches, it was estimated that this infection total is more than three times the dengue burden estimate of the World Health Organization[3]. Epidemic dengue occurs in Asia, the Americas, and some Pacific islands. The WHO regions of Southeast Asia (SEA) and the Western Pacific represent approximately 75% of the current global burden of dengue. In Asia, dengue mainly affects children, for whom it is among the 10 leading causes of hospitalization and death[4].

The pathogenesis of dengue diseases is still not clearly understood. One hypothesis concerning virus virulence has been debated with the antibody dependent enhancement hypothesis and it is generally observed that dengue diseases are uncommon in children with malnutrition. While immune deterioration was proven in patients with acquired immune deficiency syndrome (AIDS)[5-8]. Dengue virus has rarely been reported to cause uniquely prevalent, severe, or unusual disease in people infected with HIV. Moreover, no significant interactions have been documented between HIV and flaviviruses. Watt reported a transitory reduction in HIV-1 replication during co-infection with the dengue virus and

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demonstrated that samples of serum from patients in the acute phase of dengue are capable of inhibiting the in vitro infectivity of HIV-1. His attempt to identify a large number of individuals co-infected with dengue virus was unsuccessful[9]. The pathogenesis of both diseases is rather on the contrary and it is uncertain if the risk of dengue infection would be the same, greater or lower in AIDS patients in comparison to healthy individuals. Therefore, it is of great interest to study the seroprevalence of dengue virus in HIV-infected individuals.

Material and Method

This cross-sectional survey was carried out in the Pediatric Department, King Chulalongkorn Memorial Hospital. Eighty-six HIV-infected children (50 boys and 36 girls) aged under 15 years old who were treated at the HIV clinic, King Chulalongkorn Memorial Hospital and one hundred age-matched healthy children (56 boys and 44 girls) aged less than 15 years who attended an out-patient department were recruited on a voluntary basis after their parents had provided written informed consent. Those who were immunocompromised were excluded. Immuno-compromised conditions included patients who had received immunosuppressive agents e.g. radiation, corticosteroid of greater than physiologic dosage (more than 12.5 mg/m^2/day or 0.5 mg/kg/day) for more than one month, in the case of systemic lupus erythematosus (SLE) and nephritic syndrome, cytotoxic drugs e.g. cyclophosphamide, cyclosporine, patients who received chemotherapy e.g. infiltrative and hematologic diseases (leukemia, hepatoblastoma, neuroblastoma and others) for more than one month, patients who underwent post organ transplantation e.g. liver transplantation, bone marrow transplantation that received immunosuppressive agents e.g. cyclosporine, cyclophosphamide, busulfan for more than one month and uremia from chronic renal failure.

All subjects' parents were interviewed with structured questionnaires to collect details about their children's age, sex, place of birth and underlying disease. HIV-infected children were then classified according to the CDC 1994 classification[10] and clinical data were collected.

Blood sampling

A 5 ml sample of venous blood was obtained and allowed to clot for four hours at room temperature. It was then centrifuged and the serum was stored frozen at -20°C until testing. Sera were tested by plaque reduction neutralization test for neutralizing antibodies to DEN 1, 2, 3, and 4 as described[11]. After counseling, written informed consent was obtained and anonymous anti-HIV antibodies were tested among healthy children.

Serological definitions of dengue virus[12]

Levels of neutralizing antibody (Nab) were measured by using the 50% plaque-reduction neutralization test (PRNT50) in LLC-MK2 cells against prototype strains of all four DVs (DVI 16007, DV2 16681, DV3 16562, and DV4 1036), Japanese encephalitis virus (JEV; Nakayama strain), and the patient's own DV isolate. We defined three patterns of plasma DV PRNT50 results: undetectable (PRNT50 <10 to all 4 DVs), monotypic (PRNT50 $\geq$10 to only 1 DV serotype) and multitypic PRNT50 pattern (PRNT50 $\geq$10 to more than 1 DV serotype). The presence or absence of PRNT50 to JEV was determined but was not used for this classification.

Data analysis

Statistical analysis was performed by using SPSS software for Windows (version 11.0; SPSS Inc., Chicago, Illinois). Demographic data (e.g. sex, age, etc.) were analyzed by calculating mean, range, and percentage and was compared using un-paired t-test or Chi-square depending on the data. Comparison of antibody between each group was done by using Pearson's Chi-square or Fischer's exact tests. Statistical significance was calculated as p-value <0.05.

Human use review and approval

The study protocol was reviewed and approved by the Ethical Review Board of Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.

Results

There were 186 children aged between 1 to 14 years and 11 months in this study. Characteristics of the study population are shown in Table 1. There were no differences in the characteristics between HIV-infected and healthy children. Most subjects in both groups came from Bangkok. All healthy children had an anonymous negative anti-HIV antibody. Most HIV-infected children were moderately immune suppressed with a variety of clinical categories (Table 2 and 3).

Fifty of eighty-six (58%) HIV-infected children and sixty-five of hundred (65%) healthy children had positive neutralizing antibodies against dengue virus by PRNT. There were no significant differences between

Table 1. Characteristics of HIV-infected children and healthy children

Characteristics	HIV-infected (n = 86)	Healthy (n = 100)	*p*-value
Age (±2 SD) (year)	7.39 (±3.76)	7.02 (±4.00)	0.382
Boy: girl	50:36	56:44	0.416
Residence	Bangkok (86.2%)	Bangkok (71%)	0.024

Table 2. Characteristics of HIV-infected children by immune and clinical status

Category	Age (year)	Boy: girl	Total No. (%)
Immune suppression			
No	7.89±2.04	12:11	23 (26.74)
Mild	7.41±4.01	18:14	39 (45.35)
Severe	7.71±3.67	16:8	24 (27.91)
Clinical			
Asymptomatic	7.65±4.30	11:11	23 (26.74)
Mild	7.76±3.64	16:9	25 (29.07)
Moderate	6.95±3.73	12:9	21 (24.42)
Severe	7.65±3.10	11:6	17 (19.77)

Table 3. Number of samples classified by HIV clinical status and immunity

Immunesuppression	Clinical (No.)				Total (%)
	N	A	B	C	
No	4	4	9	0	17/23 (74)
moderate	5	7	4	1	17/39 (44)
Severe	0	4	2	10	16/24 (67)
Total (%)	9/23 (39)	15/25 (60)	15/21 (71)	11/15 (73)	

Table 4. Comparison of seropositive (PRNT) dengue infection between HIV-infected children and healthy children

PRNT50	HIV-infected children	Healthy children
Monotypic	26	19
Multitypic	24	46
Total	50	65

p-value = 0.330

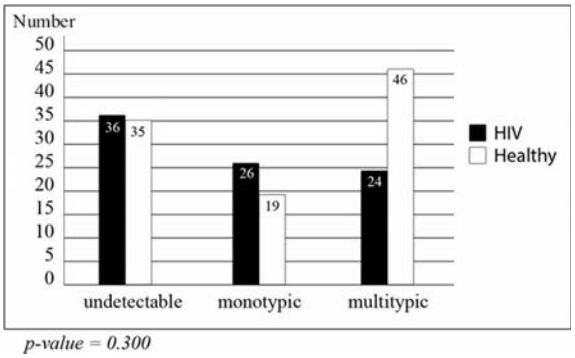

Fig. 1 Comparison of seropositive (PRNT) dengue infection between HIV-infected and healthy children.

these two groups ($p>0.05$) (Table 4 and Fig. 1). In HIV-infected children, a monotypic PRNT50 pattern was found in twenty-six children (30%) and multitypic pattern was found in twenty-four children (28%). In healthy children, monotypic PRNT was found in 19% and multitypic PRNT in 46%. Most children had neutralizing antibodies against DEN2 (Fig. 2). There

was no significant difference between these two groups.

Prevalence of seropositivity to dengue virus PRNT was significantly different between different age groups in HIV-infected children (p = 0.0140 (Table 5), while there was no difference in seropositivity to dengue virus PRNT between different age groups in healthy children (p = 0.955) (Table 6). Most healthy children were seropositive monotypic PRNT for DEN2. Table 7 shows no difference in dengue seroprevalence between HIV-infected and healthy children.

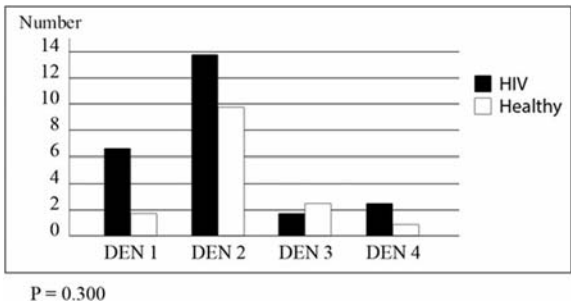

Fig. 2 Comparison of PRNT seropositive dengue virus between HIV-infected and healthy children by serotypes.

Seropositivity to dengue virus PRNT in HIV-infected children as classified by HIV clinical and immune status is shown in Table 8.

There was no difference in the average PRNT50 titer between HIV-infected and healthy children (Table 9) and in both groups, PRNT50 titers of DEN2 was found most. PRNT 50 titers of DEN1 and DEN2 were higher in HIV-infected children than healthy children. In this study, five HIV-infected children had PRNT50 titers >1,000 and were multitypic. These HIV-infected children were in A2, B1, C2, and C3, classified according to the CDC 1994 classification[10]. Three HIV-infected children had PRNT50 titers >1,000 with monotypic PRNT and were in B3 and C3, classified according to the CDC 1994 classification[10]. Furthermore, seven healthy children had multitypic PRNT50 titers >1,000 and no healthy children had monotypic PRNT 50 titers >1,000 (Table 8 and 9).

Discussion

Dengue infection is the most common arboviral infection in the world while HIV/AIDS remains a major global concern. Although the pathogenesis of both diseases affects the immune system in different manners, dengue hemorrhagic fever was proposed to

Table 5. Number of seropositive PRNT in HIV-infected children classified by age

Age	PRNT positive (No.)			Sum (%)
	No.	Monotypic	Multitypic	
<5 year	29	7	6	13 (45)
6-10 year	41	17	8	25 (61)
11-15 year	16	2	10	12 (75)
Sum	86	26	24	50 (58)

p-value = 0.014

Table 6. Number of seropositive PRNT in healthy children classified by age

Age	PRNT positive (No.)			Sum (%)
	No.	Monotypic	Multitypic	
<5 year	40	7	17	24 (60)
6-10 year	36	6	16	22 (61)
11-15 year	24	6	13	19 (79)
Sum	100	19	46	65 (65)

p-value = 0.955

Table 7. Comparison of PRNT seropositive dengue PRNT between HIV-infected children and healthy children classified by age

Age	PRNT seropositive (No.)		*p*-value
	HIV-infected children	Healthy children	
<5 year	13	24	0.622
6-10 year	25	22	0.167
11-15 year	12	19	0.860
Total	50	65	

Table 8. Number of seropositive dengue virus PRNT classified by HIV clinical status and immunity

Categories	$PRNT_{50}$ (%HIV)		
	Undetectable	Monotypic	Multitypic
Immune suppression			
No	6 (12)	7 (14)	10 (20)
Moderate	22 (44)	9 (18)	8 (16)
Severe	8 (16)	10 (20)	6 (12)
Clinical			
Asymptomatic	14 (28)	6 (12)	3 (6)
Mild	10 (20)	6 (12)	9 (18)
Moderate	6 (12)	6 (12)	9 (18)
Severe	6 (12)	8 (16)	3 (6)

Table 9. Comparison of average monotypic $PRNT_{50}$ titer between HIV-infected children and healthy children classified by dengue virus serotypes

	HIV	n	Mean	SD	t-test
DEN1	Healthy	19	24.7	77.6	0.243
	HIV	26	74.7	189.7	
DEN2	Healthy	19	147.0	206.7	0.457
	HIV	26	259.8	629.5	
DEN3	Healthy	19	41.6	101.8	0.723
	HIV	26	14.7	18.2	
DEN4	Healthy	19	9.2	13.1	0.193
	HIV	26	11.0	19.0	

be immunologically mediated[13] while HIV/AIDS causes immune suppression[8]. It is uncertain whether the risk of dengue hemorrhagic fever would be the same, greater or lower in AIDS patients. Our study showed no difference in dengue seroprevalence in both HIV-infected and healthy children although it is generally observed that dengue hemorrhagic fever is uncommon in patients with AIDS[14,15]. The seroprevalence rate of 58% in HIV-infected and 65% in healthy children in our study was rather similar to previous studies in Thailand. However, there was an increase in seropositivity in a younger age group in this study[16,17].

The lack of difference of dengue neutralizing

antibodies between HIV-infected and healthy children in this study, in addition to rare cases of dengue hemorrhagic fever in HIV-infected individuals from anecdotal records, may be explained by protective effects of immune suppression in HIV-infected persons. This may prevent them from severe dengue diseases such as Thisyakorn has reported that dengue hemorrhagic fever is rarely seen in clinically malnourished children; however, most patients with dengue hemorrhagic fever are not undernourished. Malnutrition also affects host defenses in several ways, e.g. depressed cell-mediated immunity and reduced level of secretory IgA[7]. The potential effects of HIV infection on dengue severity have yet to be determined since persons with HIV/AIDS who remain immunocompetent may pose the risk of developing severe dengue diseases[14,15].

Conclusion

HIV-infected children and healthy children had no different seroepidemiology of dengue virus infection.

What is already known on this topic?

Both dengue and HIV/AIDS are global public health problem. The pathogenesis of both diseases affects the immune system in different manners and there is no significant interaction documented between the two diseases.

What this study adds?

Seroprevalence of dengue in HIV-infected children in comparison to healthy children was studied.

The results will lead to understanding of dengue risk in HIV-infected children.

This may lead to further understanding of pathogenesis of both diseases.

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Potential conflicts of interest

None.

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ระบาดวิทยาทางน้ำเหลืองของการติดเชื้อไวรัสเด็งกีในเด็กที่ติดเชื้อเอชไอวีเปรียบเทียบกับเด็กปกติ

อุษา ทิสยากร, กัญญลักษณ์ เศรษฐไกรกุล, มาริสา เหมังกร, จุล ทิสยากร

วัตถุประสงค์: *ศึกษาระบาดวิทยาทางน้ำเหลืองของการติดเชื้อไวรัสเด็งกีในเด็กที่ติดเชื้อเอชไอวีเปรียบเทียบกับเด็กปกติ*

วัสดุและวิธีการ: *ทำการวิจัยเชิงพรรณนา ณ จุดใดจุดหนึ่งในเด็ก 2 กลุ่ม ได้แก่ กลุ่มที่ 1 เด็กติดเชื้อเอชไอวี จำนวน 86 คน โดยมีการจำแนกความรุนแรงของการติดเชื้อเอชไอวีออกเป็นระยะต่าง ๆ และกลุ่มที่ 2 เด็กปกติจำนวน 100 คน โดยเก็บพลาสมาเพื่อตรวจแอนติบอดีต่อไวรัสเด็งกีทั้ง 4 ซีโรทัยพ์ ได้แก่ DEN1, DEN2, DEN3 และ DEN4 ด้วยวิธีพลาครีดักชั่นนิวทรัลไลเซชั่น (PRNT) ในเด็กทั้ง 2 กลุ่ม*

ผลการศึกษา: *เด็กที่ติดเชื้อเอชไอวีมีผลบวกต่อไวรัสเด็งกี 50 ใน 86 คน (58%) เด็กปกติมีผลบวกต่อไวรัสเด็งกี 65 ใน 100 คน (65%) และไม่มีความแตกต่างทางสถิติของการติดเชื้อไวรัสเด็งกีในเด็กที่ติดเชื้อเอชไอวีเมื่อเปรียบเทียบกับเด็กปกติ*

สรุป: *ระบาดวิทยาทางน้ำเหลืองของการติดเชื้อไวรัสเด็งกีในเด็กที่ติดเชื้อเอชไอวีไม่แตกต่างจากเด็กปกติ*