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Increased Carotid Intima-Media Thickness in Children with a History of Dengue Hemorrhagic Fever

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Abstract. We assessed carotid intima-media thickness (cIMT) and arterial stiffness in 28 children and adolescents with previous dengue hemorrhagic fever (DHF) (mean interval between DHF and cardiovascular assessment, 8.4 years), and 34 controls in a low-resource setting. Participants with previous DHF had an adjusted increased cIMT of 42.6 μm (95% confidence interval [CI]: 10.0–75.3, $P = 0.01$), and 61.7 μm (95% CI: 21.5–102.0, $P < 0.01$) in a subgroup analysis on dengue shock syndrome. There were no differences in arterial stiffness. In this first exploratory study, children and adolescents with a history of DHF had an increased cIMT, which may be modulated by dengue severity.

INTRODUCTION

Dengue is the most common arthropod-borne viral disease. Fifty to one hundred million cases occur annually and the incidence is increasing.[1] In urban Indonesia, dengue burden is particularly high; 80% of children aged 10 years or older are seropositive.[2] Dengue is usually self-limiting but a minority develop significant plasma leakage and/or hemorrhage, leading to dengue hemorrhagic fever (DHF).[3] There are an estimated 500,000 DHF cases annually, of which 22,000 are fatal.[1]

Considerable evidence supports an association between pediatric infectious diseases and the development of cardiovascular disease in adulthood.[4] Adverse cardiovascular effects of infectious diseases are well-described in both chronic infections such as HIV, where adverse vascular changes are evident in childhood,[5] and severe acute infections. For example, a dose–response association has been reported between the number of acute childhood infection-related hospitalizations and the risk of cardiovascular disease events in adulthood.[6] The mechanism that connects an infectious disease with the development of atherosclerosis has not yet been completely elucidated, although recent evidence suggests that trained immunity, the development of macrophages with a persistent proinflammatory phenotype in response to stimulation by microorganisms, might play a significant role.[7]

The effects of infection-induced inflammation on the cardiovascular system may be most pertinent if the primary infection affects the vasculature. In DHF, endothelial dysfunction occurs leading to plasma leakage, with increased biomarkers indicative of vascular damage.[8] However, it is unknown whether this vascular damage is transient or persistent. In this study, we investigated whether previous DHF in childhood was associated with vascular parameters indicative of preclinical atherosclerosis several years later.

METHODS

We performed a cross-sectional study with 29 children previously admitted with DHF between 2009 and 2015 at a tertiary reference hospital in Jakarta, at a mean follow-up of 8.4 years post-DHF. One participant was excluded because of extensive comorbidities. In addition, 34 healthy controls were recruited by inviting cases to bring acquaintances or siblings without a history of DHF hospitalization. Exclusion criteria were ongoing chronic infectious or inflammatory disease. Because of the low-resource setting, blinding of observers for case and control status was not possible as recruitment and vascular assessment were performed by the same researchers.

Before data collection, a sample size calculation was performed using carotid intima-media thickness (cIMT) as the primary outcome parameter. Based on a study that assessed the effects of pediatric HIV on cIMT, a mean difference in cIMT of 36 μm was assumed. An assumed cIMT standard deviation (SD) of 34.1 μm was derived from a study on 204 healthy children.[5,9] Using a two-sided α of 0.05 and a β of 0.20, the required sample size was 15 participants per study arm.

The study was approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia. Informed consent was obtained from the participants' caregivers, or if they had reached the legal age of majority, from the participants.

Data on the initial DHF hospitalizations were extracted from prospectively collected patient data in 2009–2015 that included the following: 1) dengue severity by both the adjusted 1997 WHO classification (DHF grades I–IV; grades III and IV indicate dengue shock syndrome [DSS]) commonly used in Indonesia and the 2009 WHO classification; 2) date and duration of hospitalization; and 3) clinical parameters including significant plasma leakage and gastrointestinal bleeding.

Three sets of ultrasonographic measurements of the right carotid artery were performed with the Esaote MyLabOne system based on the wall-track system. The cIMT of the far wall was measured 1.5 cm caudal from the carotid bifurcation. Blood pressure was measured two to three times using an OMRON HBP-1300 automated sphygmomanometer. Values are used to estimate mean carotid pressure, assuming a constant pressure in the whole arterial tree. Measurements of average right cIMT, carotid diameter, arterial distension, and blood pressure were used to calculate the elastic properties of the right carotid artery as a hollow structure (arterial distensibility) and of the wall (elastic modulus) as previously described.[10]

All measurements were performed by a single trained researcher (T.V.). Double measurements were performed in five cases and five controls. Using a two-way mixed model,

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intra-class coefficients for cIMT and arterial distension were 0.94 and 0.91, respectively, indicating excellent intra-rater reliability.[11]

Data on possible confounders, including participant and parental smoking status, socioeconomic status, and other hospitalizations, were obtained by questionnaires administered at the cardiovascular assessment to caregivers or older participants.

Data are presented as proportions or means (SD), or in the case of a skewed distribution, as medians. Differences in characteristics between cases and controls were tested using independent sample *t*-tests, Chi-square tests, or Mann–Whitney *U*-tests, as appropriate.

To assess associations between a previous DHF episode and vascular parameters, we performed univariable and multivariable linear regression with cIMT, distensibility, and elastic modulus as dependent variables, respectively, and history of DHF as the exposure of interest. Gender and parental education were included as confounders based on observed differences and BMI *z*-score, as obesity has been previously associated with DHF risk.[12] Participant and parental smoking status were not included because of no observed group differences. Systolic blood pressure was a priori identified as a possible intermediate in the relationship between DHF and cIMT and so was added in an additional explanatory model.

A subgroup analysis using the same design and analysis was performed on children hospitalized with DSS and all controls. All analyses were performed using SPSS version 21.

RESULTS

Participant characteristics are shown in Table 1. Groups were similar in age and no participants were HIV positive. Participants with a history of DHF were more likely to be female, had a higher systolic blood pressure, and had lower self-reported overall health, and their caregivers remained in education longer.

Characteristics of the DHF hospitalizations are shown in Table 2. The majority (n = 14; 56%) met the adjusted WHO 1997 DSS definition. Most children (n = 22; 88%) suffered from plasma leakage, whereas severe bleeding (n = 3) or other complications (n = 4) were uncommon. The mean age at DHF hospitalization was 6.9 years (range 2.8–8.8 years), and the mean interval between hospitalization and cardiovascular assessment was 8.4 years (range 0.8–16.5 years).

TABLE 1
Characteristics of participants by history of DHF

Characteristics	Cases, n = 28	Controls, n = 34	P-value
Age (years, range)	15.1 (5.0–24.2)	15.0 (5.1–24.9)	0.91
Male gender (n, %)	9 (32.1%)	16 (47.1%)	0.23
Weight (kg)	51.7 (4.3)	45.7 (2.3)	0.20
Height (cm)	154.9 (2.4)	151.0 (2.1)	0.23
BMI, crude	21.0 (1.3)	19.7 (0.6)	0.35
BMI, *Z*-score	–0.1 (0.3)	–0.2 (0.2)	0.85
BMI, percentile (%)	48.7 (6.8)	48.4 (5.6)	0.97
Chest circumference (cm)	79.4 (2.7)	77.2 (1.7)	0.48
Abdominal circumference (cm)	72.2 (2.8)	70.1 (1.6)	0.50
Hip circumference (cm)	83.0 (3.0)	79.0 (1.7)	0.24
Systolic blood pressure (mmHg)	116.7 (3.2)	109.5 (2.2)	0.06
Diastolic blood pressure (mmHg)	67.1 (2.0)	64.6 (1.3)	0.29
Mean arterial pressure (mmHg)	83.7 (2.3)	79.2 (1.6)	0.10
Pulse frequency (bpm)	81.3 (2.6)	81.1 (1.9)	0.95
Right carotid arterial distension (μm)	593.5 (120.0)	592.3 (117.7)	0.97
Right carotid diameter (mm)	6.44 (0.11)	6.46 (0.06)	0.88
History of chronic infection/inflammation (n, %)	–	–	0.27
HIV/AIDS	0 (0.0)	0 (0.0)	–
Chronic infectious disease	1 (3.6)*	0 (0.0)	–
Inflammatory disease	0 (0.0)	0 (0.0)	–
None of the above	27 (96.4)	34 (100.0)	–
History of hospitalization for other causes (n, %)†	–	–	0.71
None	23 (82.1)	27 (79.4)	–
Once because of infectious disease	2 (7.1)	3 (8.8)	–
Multiple times because of infectious disease	0 (0.0)	0 (0.0)	–
Once because of other causes	2 (7.1)	3 (8.8)	–
Multiple times because of other causes	0 (0.0)	1 (2.9)	–
Multiple times because of infectious disease and other causes	1 (3.6)	0 (0.0)	–
Participant smoking status (packyears, median)	0.0	0.0	0.12‡
Parental smoking status (packyears, median)§	3.1	3.0	0.76‡
Parental early onset CVD (n, %)	–	–	0.96
None	24 (85.7)	29 (85.3)	–
Father	2 (7.1)	3 (8.8)	–
Mother	2 (7.1)	2 (5.9)	–
Total years of education			
Participant (years)	8.3 (0.8)	7.6 (0.6)	0.53
Father (years, median)	12.0	12.0	0.21‡
Mother (years, median)	12.0	12.0	0.01‡
Father and mother combined (years)	24.4 (0.9)	21.2 (1.0)	0.03

DHF = dengue hemorrhagic fever; BMI = body mass index. All values are mean standard deviation, unless otherwise indicated. Independent *t*-test and Chi-square were used as appropriate.
* Recurrent tonsillitis and asthma, 1 case. Last episode was 6 months before the current measurements.
† Excluding the hospitalization for DHF in cases.
‡ Mann–Whitney *U*-test was used because of non-normal distribution.
§ Combined amount of packyears of the parents of the participant.

TABLE 2
Characteristics of DHF hospitalization (n = 25)*

Dengue grade (adjusted WHO 1997; *n*, %)	
Dengue fever	0 (0)
Dengue hemorrhagic fever grade I	6 (24)
Dengue hemorrhagic fever grade II	5 (20)
Dengue hemorrhagic fever grade III (DSS)	7 (28)
Dengue hemorrhagic fever grade IV (DSS)	7 (28)
Dengue grade (WHO 2009; *n*, %)	
Dengue fever without warning signs	1 (4)
Dengue fever with warning signs	10 (40)
Severe dengue	14 (56)
Age at hospitalization (years)	6.9 (2.8–8.8)
Time between hospitalization and current measurements (years)	8.4 (0.8–16.5)
Duration of hospitalization (days)	4.4 (3–8)
Presence of plasma leakage (*n*, %)	22 (88)
Presence of mucosal bleeding manifestations (*n*, %)	4 (16)
Presence of gastrointestinal bleeding manifestations (*n*, %)	3 (12)
Presence of dermal bleeding manifestations (*n*, %)	12 (48)
Presence of pleural effusion (*n*, %)	4 (16)
Presence of encephalopathy (*n*, %)	1 (4)
Presence of other severe complications (*n*, %)†	3 (12)
Presence of dengue IgM antibodies (%)	56
Presence of dengue IgG antibodies (%)	80
Mean lowest recorded hematocrit (%)‡	32.6 (16.6–45.3)
Mean highest recorded hematocrit (%)‡	43.6 (30.0–56.0)
Mean Δ-hematocrit (%)‡,§	39.7 (4.9–123.8)
Mean lowest recorded thrombocyte count (cells/μL)‡	42,929 (6,000–89,000)
Mean highest recorded thrombocyte count (cells/μL)‡	98,088 (44,100–206,000)
Mean Δ-thrombocyte count (%)‡,§	225.8 (14.6–1,250.0)
Mean highest recorded leukocyte count (cells/mm^3)	13,468 (3,300–127,000)
Mean lowest systolic blood pressure (mmHg)	97.9 (70–120)
Mean lowest diastolic blood pressure (mmHg)	58.0 (32–80)
Mean lowest mean arterial pressure (mmHg)	71.3 (50–93)
Crystalloid fluid management (%)	
Maintenance (Ringer's lactate)	6 (24)
Maintenance (dextrose + NaCl)	2 (8)
10% rehydration deficit (Ringer's lactate)	17 (68)
Presence of colloid transfusion (%)	5 (20)

DHF = dengue hemorrhagic fever; DSS = dengue shock syndrome. All values are mean (range), unless otherwise indicated.

* Clinical data on the hospitalization period were not available for three participants and these are, therefore, not included in this table.

† Three of 25 participants had renal impairment as a complication of DHF.

‡ One participant had only one reported value of hematocrit and thrombocyte count and is not included in the mean data of those parameters, but is included in the other parameters.

§ Expressed as "(highest value–lowest value)/lowest value * 100%."

Carotid IMT of children with a history of DHF was significantly increased compared with controls (Table 3). This effect was more pronounced when corrected for gender, parental education, and BMI z-score (mean difference of 42.6 μm, P = 0.01). Systolic blood pressure was considered a possible intermediary, and additional adjustment for systolic blood pressure attenuated the findings slightly (mean difference of 33.2 μm, P = 0.05).

Subgroup analysis of children with DSS (n = 14) and controls showed a more pronounced difference in cIMT (adjusted mean difference: 61.7 μm, $P < 0.01$). An additional subgroup analysis was performed (Supplemental Table 1) to assess whether the length of the time interval between DHF and cardiovascular assessment affected cIMT. There was an association between a longer interval (upper meridian) and cIMT. There were no group differences in arterial stiffness parameters.

DISCUSSION

This study suggests that a childhood history of DHF is associated with an increased cIMT later in life. The current measurements were performed on average 8.4 years after the episode of DHF, indicating a persistent effect. Subgroup analysis of DSS cases showed stronger evidence of an association, possibly compatible with a relationship between dengue severity and cIMT. In addition, we found an association between a longer time interval between DHF and cardiovascular measurements and cIMT, suggesting a cohort effect. However, this finding should be interpreted cautiously because of its low power.

We observed no group difference in arterial stiffness. Interestingly, we observed some evidence of an increased systolic blood pressure in DHF cases. We hypothesized that this increase reflected a possible intermediary effect, but adjusting for systolic blood pressure in an additional model still showed a significant increase in cIMT.

An increased cIMT in childhood is considered a possible early biomarker for preclinical atherosclerosis,[13,14] although the long-term clinical implications remain unclear. Increased cIMT is increasingly recognized in children with severe infection, although these reports mainly focus on chronic infectious diseases, such as in HIV.[5] Our findings indicate that lasting vascular structural changes may also occur after a single brief intense inflammatory stimulus, such as DHF.

Certain polymorphisms of genes encoding for proinflammatory cytokines are more prevalent in DHF patients.[15] It is possible that by studying DHF cases, we have selected a population more susceptible to an exaggerated inflammatory response more generally, which could contribute to increased cIMT. Alternatively, DHF may have a pathogen-specific effect on vasculature as the magnitude of the cIMT increase was unexpected: a mean adjusted difference in cIMT of 70.4 μm has been reported in ART-naive HIV pediatric patients,[5] comparable with our mean adjusted difference of 61.7 μm in DSS.

This preliminary study has several limitations. Although the small sample size of our study allowed us to observe differences in cIMT, it may have had insufficient power to show differences in parameters of arterial stiffness. Also, the lack of blinding might have introduced bias, although the automatic wall-track system minimizes this possibility.

Our findings highlight a possible effect of DHF in childhood on cardiovascular disease. Dengue hemorrhagic fever seems to be one of several infectious diseases that are linked with vascular structural changes and is, to our knowledge, one of the first non-chronic infectious diseases described in this context. Our study is one of the few long-term follow-up studies performed on patients with DHF. There are reports of dengue-related symptoms months after the episode,[16] and

TABLE 3
Association between previous hospitalization because of DHF and cardiovascular parameters, full and subgroup analysis

		Mean difference (μm, [95% confidence interval])					
		Crude	*P*-value	Adjusted #1†	*P*-value	Adjusted #2‡	*P*-value
Full analysis							
cIMT (μm, [SD])							
DHF+	429.9 (65.0)	38.9 (7.4–70.4)	0.02*	42.6 (10.0–75.3)	0.01*	33.2 (0.5–65.9	0.05*
Controls	390.9 (58.8)						
Carotid distensibility (MPa^{-1}, [SD])							
DHF+	46.2 (13.3)	−3.5 (−10.1 to 3.2)	0.30	−3.0 (−10.0 to 4.0)	0.39	–	–
Controls	49.7 (12.8)						
Carotid elastic modulus (kPa, [SD])							
DHF+	373.5 (192.2)	9.4 (−70.8 to 89.5)	0.82	2.7 (−79.6 to 85.0)	0.95	–	–
Controls	364.2 (120.9)						
Subgroup analysis on DSS (n_{cases} = 14)§							
cIMT (μm, [SD])							
DSS+	447.9 (69.1)	57.0 (17.4–96.5)	0.01*	61.7 (21.5–102.0)	< 0.01**	53.5 (15.5–91.6)	< 0.01**
Controls	390.9 (58.8)						
Carotid distensibility (MPa^{-1}, [SD])							
DSS+	48.2 (12.5)	−1.5 (−9.6 to 6.6)	0.72	−1.5 (−9.9 to 6.9)	0.73	–	–
Controls	49.7 (12.8)						
Carotid elastic modulus (kPa, [SD])							
DSS+	315.8 (89.6)	−48.4 (−120.6 to 23.8)	0.18	−54.4 (−128.0 to 19.1)	0.14	–	–
Controls	364.2 (120.9)						

cIMT = carotid intima-media thickness; DHF = dengue hemorrhagic fever; DSS = dengue shock syndrome; SD = standard deviation.
* P value of less than 0.05 but more than 0.01 (denotes significance).
** P value of less than 0.01.
† Adjusted model corrected for gender, BMI *z*-score, and combined educational years of parents as a proxy for socioeconomic status.
‡ Adjusted model, similar to model 1, additional correction for systolic blood pressure. This model was not used for carotid distensability and elastic modulus, as systolic blood pressure is a part of the composition of these parameters.
§ Including only cases that have been hospitalized for DSS (DHF grade III or IV, *n* = 14) according to the adjusted WHO 1997 definition, and all healthy participants.

calls for more research on possible long-term effects of dengue have already been made.[17]

In conclusion, our findings suggest that DHF in childhood may be associated with increased cIMT, and the effect may be modulated by DHF severity.

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Note: Supplemental table appears at www.ajtmh.org.

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