Capillary Leakage in Travelers with Dengue Infection: Implications for Pathogenesis

Eyal Meltzer, Zahava Heyman, Hanna Bin, and Eli Schwartz*

Center for Geographic Medicine and Department of Medicine "C", Department of Imaging, and the Central Virology Laboratory, and Ministry of Health, Chaim Sheba Medical Center, Tel Hashomer, Israel; Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel

Abstract. Dengue hemorrhagic fever is characterized by the presence of a capillary leak syndrome. Its pathogenesis is presumed to differ from that of classical dengue fever (DF) and to be associated with secondary dengue infection. Returning travelers given a diagnosis of DF were evaluated for capillary leakage with abdominal sonography. Data were compared between travelers with primary/secondary infection defined by epidemiologic and serologic parameters. A total of 12 (34.3%) of 35 patients had sonographic signs of capillary leakage. Most (85%) patients with capillary leakage had classical DF. Capillary leak was diagnosed in 32% of primary dengue cases and in 40% of secondary dengue cases ($P = 0.69$). The two patients given a diagnosis of dengue hemorrhagic fever had primary infections. The high prevalence of capillary leakage among travelers, most of them with primary exposure to dengue, calls into question the importance of secondary infection in causing capillary leakage in dengue infection.

INTRODUCTION

Dengue viruses (DENVs) are the most prevalent of human arboviral infections, with 50–100,000,000 annual cases.[1] Dengue hemorrhagic fever (DHF) and dengue shock syndrome are severe clinical forms of dengue fever (DF). First recognized in the 1950s, DHF has been increasingly reported in absolute numbers and the number of countries affected. Concomitantly, DF and DHF have been increasingly reported among travelers.[2]

Until recently, the prevailing medical practice (as was expressed in the World Health Organization [WHO] 1997 guidelines) has adopted a dichotomous view of dengue infection, distinguishing between a non-severe simple/classic DF and DHF/dengue shock syndrome that were defined by the presence of a bleeding phenomena, severe thrombocytopenia, and a capillary leak syndrome.[3] Although the definition of severe dengue in the recent 2009 WHO guideline was expanded to include severe organ dysfunction and bleeding *per se*, it is still capillary leakage leading to fluid accumulation that defines most cases of severe dengue.[4]

The prevailing theory of DHF is that the pathogenesis of DHF differs from that of classical DF. Dengue hemorrhagic fever is presumed to be caused by secondary dengue infection and mediated by antibody-dependent enhancement (ADE) of dengue infection. This theory has been increasingly challenged in recent years as the main explanation of severe dengue because the disease is seen more as a spectrum of severity with a complex, multifactorial pathogenesis.

Thoraco-abdominal ultrasound is a highly accurate method for assessing even small amounts pleural effusion/ascites, and has a sensitivity of nearly 100%.[5] Thus, sonography is a suitable tool for assessing capillary leakage in dengue patients, and is recommended by the 2009 WHO guidelines. Travelers are an excellent study population for the suggested association of secondary infection with capillary leak because most originate from non-dengue endemic areas (such as Israel), and for most of them the dengue attack is their primary infection. Our aim was to prospectively evaluate returning travelers with dengue infection for evidence of capillary leak, and provide a correlate with previous travel history and immune status.

METHODS

Study design: a prospective observational study. Sheba Medical Center diagnoses a significant proportion of all DF cases in Israel, and is associated with the Israeli National Virological laboratory, which is the national center for arbovirus testing. The study was conducted during January 2002–January 2009. Patients were Israeli travelers returning from tropical destinations to which dengue virus is endemic with a febrile illness. Patients were evaluated for various causes of post-travel fever, including malaria. Cases of dengue were diagnosed clinically and were confirmed serologically. The serologic tests for DENVs were two ELISAs: a dengue capture IgM and a dengue IgG indirect test (Panbio, Brisbane, Queensland, Australia).

Dengue hemorrhagic fever was defined according to WHO criteria.[3] Patients given a diagnosis of DF were routinely followed-up for capillary leakage and other complications by physical examination, laboratory tests (including a complete blood count and a chemistry panel on admission repeated daily afterwards), and by abdominal sonography.

Ultrasound was performed by using Acuson Sequoia 512 and curvilinear (2–6 MHz) and vector (2–4 MHz) transducers. Sonographic evidence of capillary leakage was considered to be present if either of ascites, pleural effusion, or a thickened, edematous gallbladder were present. All travelers returning from their first trip to a dengue-endemic region were considered to have a primary dengue infection. Travelers with more than one trip to a dengue-endemic region were differentiated to primary/secondary dengue infection according to kit manufacturer's instructions. A IgG result > 40 Panbio units in an acute-phase serum sample, which is four times above the cutoff level (equivalent to hemagglitination inhibition assay of 2,560) is considered a secondary infection.

Statistical analysis included the Fisher exact test for categorical parameters and the Student *t*-test for continuous parameters. The study was approved by the institutional review board at the Sheba Medical Center.

RESULTS

During the study period, 35 hospitalized patients were given a diagnosis of dengue infection at the Sheba Medical Center. The yearly number varied from 2 to 10 patients (mean ± SD = 4.8 ± 2.8 patients/year), and 2008 was the year with the highest

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*Address correspondence to Eli Schwartz, Center for Geographic Medicine and Department of Medicine C, The Chaim Sheba Medical Center, Tel Hashomer, 52621 Israel. E-mail: elischwa@post.tau.ac.il

number of patients. Patients were hospitalized mostly within 3 days of their return from abroad (range 1–7 days, mean ± SD = 3.5 ± 2.1 days).

Patient demographic details are seen in Table 1. Most cases were infected while traveling to Asia, with 68.6% acquired in Southeast Asia (mostly Thailand), and an additional 22.9% were acquired in India. In 13 (37.1%) of cases, no previous travel to dengue-endemic regions was reported. The rest of the patients had traveled at least once before to dengue-endemic regions. None of them had recalled having had a clinical disease compatible with dengue during their previous travel. Among repeated travelers, serologic tests showed primary infections in 12 of 22 cases; overall 71.4% of cases were defined as primary dengue and 28.6% as secondary dengue.

Two patients (6.0%) fulfilled the WHO definition of DHF; both had primary infection with sonographic evidence of capillary leak, one had melena and the other had metrorrhagia. None of the patients had significant (> 20%) hemoconcentration. All 35 cases recovered uneventfully, and no severe complications of dengue (e.g. shock, major bleeding, encephalitis or acute myocarditis) were documented.

Sonography was performed a mean ± SD = 6.9 ± 1.0 days after onset of fever. Overall, 12 (34.3%) of 35 travelers had signs of capillary leak (10 cases of ascites, with concomitant gallbladder wall edema in 3 cases and concomitant pleural effusion in 5 cases, and another 2 cases of isolated pleural effusion). Among primary dengue, 8 (32.0%) of 25 cases were diagnosed with capillary leakage, and among secondary dengue 4 (40%) of 10 patients had these findings. However, this difference was not statistically significant (P = 0.69). Serum protein levels were significantly lower in the capillary leakage group (P = 0.006). The male/female ration was lower in this group, but this difference did not reach statistical significance (P = 0.07); all other clinical, hemodynamic, and laboratory parameters were similar among patients with or without sonographic evidence of capillary leak (Table 2).

Both cases of DHF were diagnosed as primary infections. Along with severe thrombocytopenia (platelets counts = 17,000 and 34,000 × 10^6/L) and bleeding manifestations, both DHF cases had sonographic evidence of ascites, and there was concomitant pleural effusion in one case.

DISCUSSION

Dengue infection is caused by a group of four closely related viruses. These relatively strict human pathogens may have evolved from closely related simian relatives as late as 800 years ago.[6] Outbreaks of dengue-like illnesses have been reported for more than two centuries in subtropical and tropical countries. However, outbreaks of severe and fatal dengue, i.e., DHF, were only recognized in the latter half of the 20th century. It should be noted that virologic and serologic tests confirming DENV as the pathogen were in fact only introduced in the 1960s.

Despite its high prevalence, much is still unclear about the pathophysiology of dengue infection, mainly because of the lack of an adequate animal model. It is usual in standard medical texts to find classical DF and DHF to be discussed separately as two distinct nosological entities.[7–9] Capillary leakage is considered to be the primary lesion that underlies DHF, and was essential to the WHO definition of DHF.

Thoraco-abdominal sonography with detection of clinically inapparent ascites, pleural effusions, or gallbladder wall edema detects positive findings in most DHF cases investigated in dengue-endemic countries.[10] Its use has never been assessed in travelers, a population that is mostly naive to dengue infection, and most cases undoubtedly are primary dengue infection. We have shown that among returning travelers with mostly classical DF, capillary leakage occurs in 34.3% of cases. Furthermore, we have also shown that there is no significant difference between the incidence of capillary leakage between persons with primary and secondary infections.

Studies in India have demonstrated that among hospitalized patients with uncomplicated DF, the prevalence of sonographic capillary leak is high and ranges from 46%[11] to as high as 100%.[12] Since in resource-poor environments only severe

TABLE 1
Demographic, clinical, and laboratory characteristics in travelers diagnosed with dengue infection*

Characteristic	All travelers (n = 35)	Primary infection (n = 25)	Secondary infection (n = 10)	P
Epidemiologic data				
Mean ± SD age (years)	30.8 ± 10.6	31.2 ± 11.4	30.8 ± 9.3	0.92
M/F ratio	1.5	2.1	1.0	0.44
Travel destination, %				
Southeast Asia	68.6	76.0	50.0	0.22
India	22.9	20.0	30.0	0.66
Latin America	9.6	4	20.0	0.08
Mean ± SD time from return to admission (days)	3.5 ± 2.1	3.3 ± 1.9	4.0 ± 2.6	0.42
Clinical and laboratory data, mean ± SD				
Fever duration on admission (days)	4.5 ± 2.0	4.5 ± 2.2	4.5 ± 1.9	0.97
Fever duration on day of sonography (days)	6.9 ± 0.9	6.9 ± 1.0	7.0 ± 0.5	0.71
Systolic BP (mm of Hg)	107.8 ± 14.7	107.5 ± 15.0	108.5 ± 15.0	0.87
Diastolic BP (mm of Hg)	65.0 ± 7.8	64.6 ± 7.5	65.7 ± 8.9	0.74
Leukocyte count (×10^6/L)†	3,001 ± 1,024	2,986 ± 1,145	2,917 ± 664	0.76
Neutrophil count (×10^6/L)†	1,287 ± 433	1,382 ± 498	1,107 ± 252	0.11
Platelet count (×10^6/L)†	93 ± 46	93 ± 41	98 ± 55	0.86
Hematocrit on admission (%)	43.9 ± 3.3	41.8 ± 3.6	41.7 ± 2.8	0.89
Hematocrit after 1 day (%)	40.9 ± 2.9	41.1 ± 3.1	40.7 ± 2.7	0.76
Total serum protein (g/dL)†	6.8 ± 0.6	6.8 ± 0.5	6.8 ± 0.5	0.85
Sonographic data				
Sonographic evidence of capillary leak, n/N (%)	12/35 (34.3)	8/25 (32)	4/10 (40)	0.69

*BP = blood pressure.
†Minimum laboratory values.

TABLE 2
Comparison of dengue cases with/without sonographic signs of capillary leak*

Characteristic	Capillary leak (n = 12)	No capillary leak (n = 23)	P
Epidemiologic data			
Mean ± SD age (years)	31.1 ± 10.7	31.1 ± 11.2	0.99
M/F ratio	0.50	2.3	0.07
Travel destination (%)			
Southeast Asia	72.3	69.5	1.0
India	18.2	26.1	1.0
Central America	9.1	4.3	1.0
Mean ± SD time from return to admission (days)	3.2 ± 2.4	3.4 ± 2.0	0.72
Clinical and laboratory data, mean ± SD			
Fever duration on admission (days)	4.5 ± 1.7	4.3 ± 2.3	0.92
Fever duration on day of sonography (days)	6.8 ± 0.4	6.9 ± 1.0	0.69
Systolic BP (mm of Hg)	105 ± 16	110 ± 14	0.38
Diastolic BP (mm of Hg)	62 ± 6	67 ± 8	0.14
Leukocyte count ($\times 10^6$/L)†	3,032 ± 1,098	2,934 ± 1,040	0.60
Neutrophil count ($\times 10^6$/L)†	1,213 ± 518	1,325 ± 387	0.53
Platelet count ($\times 10^6$/L)†	82 ± 61	100 ± 36	0.15
Hematocrit on admission (%)	41.4 ± 3.5	42.0 ± 3.1	0.82
Hematocrit after 1 day (%)	40.3 ± 3.4	41.2 ± 2.6	0.35
Total serum protein (g/dL)†	6.3 ± 0.7	7.0 ± 0.5	0.006

*bp = blood pressure.
†Minimum laboratory values.

clinical cases of dengue may be referred to a hospital, a significant selection bias may account for the prevalence of capillary leak in classical DF. Additional issues such as the contribution of child malnutrition to hypoproteinemia and capillary leakage also raise doubts over the true incidence of capillary leak in DF. Yet our results have reproduced a high prevalence of capillary leakage in healthy young adults with classical DF. Our series is also hospital based and therefore a selection bias may also be present. However, such a selection bias is in all probability far less pronounced among returning travelers in Israel because universal health insurance coverage exists and therefore many post-travel febrile cases will be hospitalized for investigation.

In recent years, even the clinical usefulness of the WHO definition of DHF has been called into question.[11] The recently modified WHO definitions have retained capillary leakage, defined as fluid accumulation with/without shock, as the defining lesion of most cases of severe dengue (with the rest being rare, severe complications of dengue such as major bleeding *per se*, encephalitis, or myocarditis).[4]

The fact that more than one-third of our travelers were diagnosed with capillary leakage during an apparently non-severe clinical illness raises questions regarding the usefulness of even the new WHO definitions. Our results suggest that capillary leakage may occur in most DENV infections and rarely lead to clinically apparent phenomena, severe disease with shock and hemorrhage, frequently discernible by sonography, and perhaps universal at the microscopic level.

It is interesting to note that in this respect a recent trial among U.S. military personnel was conducted as part of the dengue vaccine development program. Sonography demonstrated capillary leakage in 7 (58.3%) of 12 of DENV-infected volunteers. None of these cases were clinically defined as DHF, but several volunteers were completely asymptomatic.[13]

The mechanism that underlies capillary leakage in DF remains as yet undefined. The main debate is centered on the role of ADE, i.e., immune enhancement of viral replication because of previous exposure to DEN-V as a cause of capillary leakage. Theoretically, a comparison of the prevalence of capillary leakage in unexposed and previously exposed populations would have definitively answered this question. However, such a study is not possible in areas where dengue is already established. Differentiating primary versus secondary infection based on serology tests is subject to errors because cross-reactivity with other flaviviruses is common.[14] Thus, the presence of IgG against Japanese encephalitis, West Nile, or other flaviviruses may lead to the false diagnosis of secondary infection in patients with primary DF.

Travelers from non-endemic countries are an ideal study population because they are generally healthy, well-nourished young adults, without previous exposure to dengue. Several previous studies have reported on the clinical and epidemiologic features of DF/DHF in travelers and have shown that the prevalence of DHF among travelers is at least in the region of 1–3%, a proportion that is similar to that seen in disease-endemic populations.[15]

The issue of primary versus secondary infection was not extensively studied in travelers with DHF. In our series, capillary leakage was found in 32% of cases with primary infection, and the two cases of DHF were primary infections. A few published case reports of travelers with DHF have included data on primary and secondary dengue.[16–19] Together with our cases, 8 (88.9%) travelers of 9 with DHF had a primary infection. Thus, it appears likely that capillary leak in DENV infection is not the result of ADE caused by secondary exposure. Capillary leak is more likely to represent a fundamental mechanism of disease in DEN-V infection, regardless of the host immune status.

It should be noted that in all likelihood, the prevalence of secondary infection in our series is overestimated. Among travelers, the differentiation between primary and secondary infection may be hampered by cross-reactivity to administered flavivirus vaccines (yellow fever and Japanese encephalitis vaccines).[20] However, this finding could only have led to an overestimation of the role of secondary infection in causing capillary leak in travelers, which further strengthens our findings, and that secondary infection is not a prerequisite condition for development of capillary leak in dengue infection and does not increase the risk for severe disease among travelers with dengue infection.

More than 30 years ago, the president of the American Society of Tropical Medicine and Hygiene, the late Leon Rosen, lamented the universal acceptance of the immune enhancement theory as established fact, despite the existence of no valid data to support such a contention.[21] It is safe to summarize, that the data on DENV infection among travelers also calls into question the importance of immune enhancement caused by secondary infection as the cause of capillary leakage in DF. Although existence of the laboratory phenomenon of ADE in dengue is well documented,[22] a renewal of the long abandoned debate regarding its clinical significance is well merited. Furthermore, the recent redefinition of severe dengue creates a need for a retesting of the purported association between secondary infection and severe dengue.

The significance of these issues is beyond a pure academic debate because the wide acceptance of the immune enhancement theory during the past 40 years may have contributed to the delay in the production of a dengue vaccine because of fears of promoting rather than preventing DHF.[23,24] This delay is highly unfortunate because only the introduction of a dengue vaccine is likely to significantly lessen the incidence of dengue in disease-endemic regions, decrease travel-related circulation of DENV, and perhaps prevent an explosive introduction of dengue to new regions.

Capillary leakage is prevalent in dengue-infected travelers; most have a clinically mild infection. Therefore, the accepted correlation between the existence of capillary leakage and severe dengue is called into question. Furthermore, capillary leakage is prevalent in dengue-infected travelers that have had only a single exposure to the virus. This finding calls into question the validity of the immune-enhancement theory of DHF/severe dengue.

Authors' addresses: Eyal Meltzer and Eli Schwartz, Center for Geographic Medicine and Department of Medicine C, The Chaim Sheba Medical Center, Tel Hashomer 52621, Israel, E-mails: emeltzer@post.tau.ac.il and elischwa@post.tau.ac.il. Zahava Heyman, Department of Imaging, The Chaim Sheba Medical Center, Tel Hashomer 52621, Israel, E-mail: Zehava.Heiman@sheba.health.gov.il. Hanna Binn, Central Virology Laboratory, Ministry of Health, The Chaim Sheba Medical Center, Tel Hashomer 52621, Israel, E-mail: Hanna.Bin@sheba.health.gov.il.

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