Original Article

Endemic Dengue Fever: a seldom recognized hazard for Pakistani children

Fahad Javaid Siddiqui, Syed Rizwan Haider, Zulfiqar Ahmed Bhutta

Department of Paediatrics, Aga Khan University

Abstract

Background: Dengue fever (DF) has gained prominence as an epidemic disease in Pakistan in the recent years. However, little data exists to show its likely endemic nature.

Methodology: We retrospectively analyzed blood for dengue IgM on samples obtained during a community-based surveillance for febrile illnesses in two slum areas of Karachi, Pakistan, between June 1999 and December 2001. In this period, no epidemic of DF occurred in the city. Participants were children older than16 years who had fever $\geq 38^{\circ}$C for more than 72 hours and in whom other common infections were excluded, based on clinical examination and laboratory tests (blood culture, urinalysis, complete blood count, Typhidot$^{\circledR}$ test and peripheral blood film for malaria).

Results: One hundred and fourteen blood samples were analyzed for dengue IgM ELISA, out of which 54 (47.4%) tested positive. The incidence of DF in this community was possibly as high as 185 (95% CI: 145 – 242) per hundred thousand population/year. Older children (10 – 15 years) appeared 5.5 times more likely to be affected than their younger (0 – 5 years) counterparts.

Conclusions: DF is probably endemic in children in slums of Karachi, and likely to have high incidence rate. Older children are more susceptible to the disease. Further prospectively designed research is needed to confirm these findings. Until that time, DF may be included in the differential diagnosis of fever without focus in children in Karachi slums even in non-epidemic periods.

Introduction

Dengue fever (DF) is an arthropod-borne disease caused by any of the four serotypes of dengue virus (DEN), a member of the family *Flaviviridae*. The principal vector for DEN is *Aedes aegypti*, a highly urbanized, daytime biting mosquito that breeds in stored water. Most infections with DEN result in a benign febrile illness with headache, myalgias, thrombocytopenia, and possible hemorrhagic manifestations. Occasionally DF may progress to more serious forms of disease, namely, the dengue hemorrhagic fever (DHF) and the dengue shock syndrome (DSS), both of which can lead to death. Case fatality of DHF is reported to be as high as 5% (http://www.cdc.gov/ncidod/dvbid/dengue/facts.htm).

According to World Health Organization (WHO) estimates, about two fifths of the world population is at risk of DEN. Worldwide, 50 million DEN infections occur every year (http://www.who.int/mediacentre/factsheet/fs117/en/)and over 100 tropical and subtropical countries are reported as dengue endemic [1, 2].

DF outbreaks have been reported from Pakistan in 1994, 1995, and 1997 [3-5]. Thereafter, few sporadic cases were reported until the winter of 2006 when an outbreak occurred, followed by another from September to November in 2007 that caused significant morbidity and mortality. Evidence suggests that the overall burden of disease, as well as its severity, is on the rise in Pakistan [6-8]. Several investigators have reported hospital-based data on DF which was collected during outbreaks of the disease [3-5]; however, no community-based data is available from Pakistan. In view of the serious implications of the disease and its recent epidemics in the country, it is imperative to evaluate the disease burden through community-based studies. Furthermore, it is not fully appreciated that in addition to periodic outbreaks, DF may also exist as an endemic disease in Pakistan. Interestingly, no outbreak was reported during the period of our study, from June 1999 to December 2001, giving us the opportunity to examine the likely endemic nature of DF. The aim of our work therefore was to determine whether DF exists as an endemic

disease, and to estimate its burden in the studied population.

Materials and Methods
Study design

For the purpose of a prior investigation on surveillance of febrile illnesses, we had established one study center in each of two adjacent slum areas (Sultanabad and Hijrat Colony) in the district south of Pakistan's largest city, Karachi [9]. Detailed demographic information about the area was available from surveys conducted by the department of Community Health Sciences of the Aga Khan University, Karachi, for its various projects in this locality. These two squatter settlements are located on each bank of a 30 m wide open sewer that drains liquid waste from a large part of the center of the city. Inhabitants are mostly from the northern region of Pakistan who settled here for economic reasons. Most belong to a low socio-economic class and are native speakers of the Pashtu and Hindko languages. The total population of children under the age of 16 years in the study population was 11,668.

During the period from June 1999 to December 2001, fortnightly surveillance of febrile illnesses at the community level was done with the help of a medical officer and five trained community health workers (CHW). On each visit, a CHW identified febrile children and advised their parents/guardians to bring the child to the study center. At the center the children were screened by a doctor (through a detailed history and physical examination) and those fulfilling the inclusion criteria (given below) were recruited into the study and subjected to blood tests to look for common infections in the area. After requisite tests, the remaining sera were stored at -70 °C.

Inclusion criteria

Children younger than 16 years of age with fever $\geq 38^{o}$C for 72 hours or more in the absence of clinically detectable focus of infection.

Laboratory procedures

Following informed consent, blood samples were obtained from patients fulfilling the inclusion criteria, on their first visit to the study center. In all cases, blood cultures using Bactec® system (Becktin Dickinson, USA), a complete blood count, peripheral blood smear for malarial parasite, Typhidot® – ELISA (Malaysian Biodiagnostic Research Sdn. Bhd,

Selangor Darul Ehsan, Malaysia), and urinalysis were obtained. The tests were conducted at the main clinical laboratory of the Aga Khan University Hospital, Karachi. Later in June 2003, Dengue IgM-ELISA DF Tests (Diagnostic Automation, Inc., Calabasas, CA, USA) were performed on stored sera.

Statistical analysis

Data were entered and cleaned using Epi Info version 6.04 (Centers for Disease Control and Prevention, Atlanta, GA, USA). Analysis was done using SPSS version 11.0 ® (SPSS Inc. Chicago, Illinois, USA). Incidence calculations were made using Microsoft Excel 2000 (Microsoft Corp., Redmond, WA, USA) spreadsheets. We estimated the incidence rate of DF by dividing the number of serology-positive cases by person years of follow-up expressed per 1,000. Person years were calculated by multiplying the total number of children, or number of children in each age group, by 2.5 (duration of study in years) assuming a steady state. Confidence intervals (CI) for incidence were calculated by treating the numerator as a Poisson variate and then obtaining the 95% CI for the count and then dividing these two values by person-time (http://www.stata.com/statalist/archive/2003-07/msg00875.html). Relative risks were calculated by considering the youngest age group as a reference category and, using EpiInfo, relative risks and their 95% CI were obtained.

Results
Distribution of febrile cases

A total of 17,485 febrile children were identified in the community through field visits by our teams (Figure 1). Of these, 4,198 (24%) patients had fever $\geq$ 72 hours duration, and were therefore encouraged to visit the study centre for further evaluation and management. Among this group, 63.4% (2,662) were in the age group 0-5 years, 23.9% (1,001) in the age group 5-10 years and 12.7% (535) in the age group 10-15 years.

Only 1,248 (30%) of the 4,198 patients identified by field visits actually reported to our study center and were examined by our doctor. Of these 1,248 patients, 907 patients had localizing signs of other diseases, such as, pharyngitis/tonsillitis, otitis media, pneumonia, gastroenteritis, urinary tract infection, dental infections, and skin and soft tissue infections.

Patients without clinically detectable focus of infection
Three hundred and forty one patients did not have a clear-cut focus of infection and therefore were recruited into the study and were subjected to diagnostic pathological tests (complete blood count, blood culture, peripheral blood film for malarial parasite, Typhidot® test, and urinalysis). Blood tests showed typhoid fever to be the most common infection detected in 216 (63.3%) patients, followed by other infections (Figure 1). The remaining 114 serum samples that were negative for other infections, thus labeled as undetermined viral illnesses, were tested for dengue IgM ELISA. Out of these 114 samples, 54 (47.4%) tested positive for DF. Thirty patients (55.5%) were girls.

Incidence of DF
The incidence of DF in children was found to be 185/100,000 population per year. In the sub-group of older children aged 10-16 years, the incidence was 570/100,000 population per year.

Table 1 shows overall and age-wise incidence of DF and relative risks of groups as compared to the youngest group. Figure 2 shows the age distribution of children with fever without focus, and those among them who had positive anti-dengue IgM antibodies. The commonest clinical feature of DF was abdominal pain followed by vomiting, diarrhoea and cough (data not shown).

DEN transmission was high in the study area from mid 1999 and remained so until the middle of 2000. Afterwards, the number of cases decreased until the end of study. As shown in Figure 3, there does not appear to be any relationship between the number of DF cases and either environmental temperature or rainfall.

Discussion

A number of hospital-based reports have been published on the recent epidemics of DF in Pakistan [6-8, 10,11]. Our study is the first from Pakistan to report community-based data on DF obtained during non-epidemic times. This study highlights that not only could DF be endemic, but it might be responsible for a significant number of cases of acute undifferentiated fever in this population. The endemic nature of DF in Karachi has been suggested previously [11], and our study adds weight to this postulation.

This study has revealed a high incidence of DF of 185/100,000 population per year. The figure springs to 570/100,000 population per year for children older than 10 years. Though no local figures are available for comparison, the incidence found in our study appears fairly high. This is not surprising, as DEN is known to be a major cause of acute undifferentiated fever in other endemic regions; a study from Vietnam [12] found that DF was responsible for 33.6% cases of acute undifferentiated fevers. Another study from Karachi found DF to be responsible for 26% of undifferentiated fevers in children [13]. The vast majority of DF cases may be overlooked in the community because the disease does not figure as a diagnostic possibility during non-epidemic periods. Even in our study, the samples that turned out to be dengue positive would have been added to the list of "non-specific viral illnesses" if we had not particularly tested for DF.

Our figures of disease burden come from a study conducted in the community hence reducing much of the selection bias that affects the results of hospital-based studies. Yet still, we have done augmented passive surveillance while operating through a study center and not at the household level. Consequently, we were able to screen only one third of the originally identified high-grade febrile episodes while the rest might have sought care through a private practitioner or traditional healer. This phenomenon always occurs while investigating infectious diseases through passive surveillance [14]. In addition, due to the limited availability of test kits, blood tests for DEN antibody could not be conducted on all patients with fever without focus; only those who had tested negative for other common infections were investigated. This could be the reason for our inability to find any case of dual infections with either malaria or typhoid, an occurrence that has been reported by other investigators [8,15,16].

Our cut-off period of at least 72 hours' fever meant that many patients were seen fairly early in the course of the disease. DEN serology testing at this stage may potentially give false-negative results [17]; however, the effect is likely to be small as another study from our institution showed that in the majority of cases, DF diagnosis was possible by IgM ELISA testing within 3 days of onset of symptoms [11]. Delayed serological testing of the stored sera might have caused some additional negative results.

Figure 1. Patient Flow Diagram: Distribution of identified febrile episodes and their diagnoses
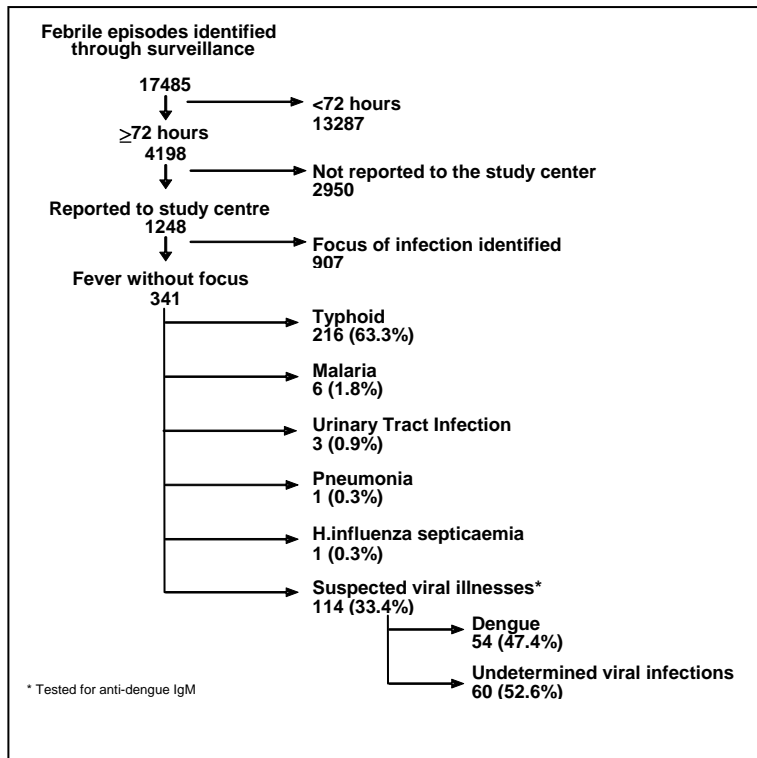

Figure 2. Age distribution of children with fever without focus in two slum areas of Karachi. Also shown is the distribution of 54 children with positive anti-dengue IgM antibodies.
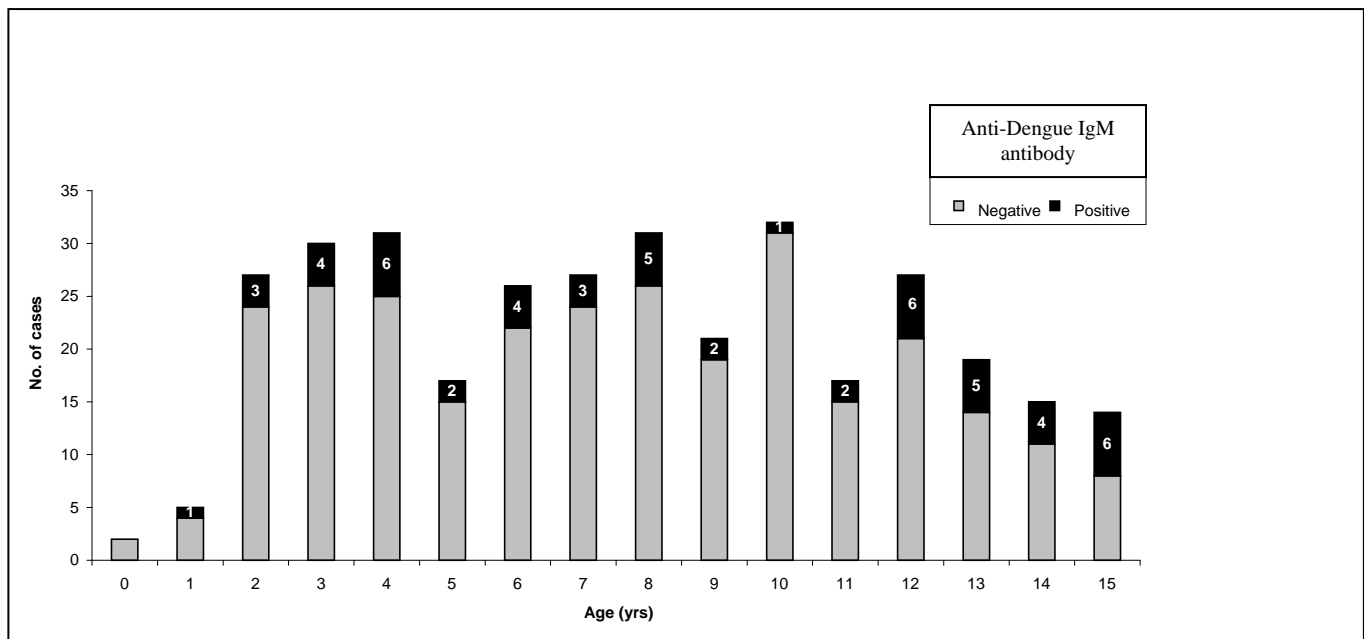
Table 1.. Incidence rate (per 1000) and relative risk with 95% confidence intervals of DF in slums of Karachi, Pakistan (Jun 99 – Dec 01)

Age groups	No. of children	Person years	Positive	IR	95% CI for IR		RR	95% CI for RR	
					Lower	Upper		Lower	Upper
0-5	6218	15545	16	1.0	0.6	1.7	1.0		
>5-10	3838	9595	15	1.6	0.9	2.6	1.5	0.75	3.1
>10-15	1612	4030	23	5.7	3.6	8.6	5.5	2.9	10.5
Overall	11668	29170	54	1.9	1.4	2.4			

IR, incidence rate; RR, relative risk; CI, confidence interval

For these reasons, therefore, it is quite likely that estimated incidence may be an underestimate of the true DF incidence. In this scenario it becomes essential to design larger, prospective studies to obtain more precise estimates of disease burden.

Our finding that children older than 10 years are more likely to get the disease is also supported by a study done in India [18]. One possible reason for higher incidence in this age group could be that repeated DEN infections lead to sequential augmentation of the immune system [1] so that the severe infections, more probable in older children, are more likely to come to medical attention as opposed to milder ones in the younger age group. However, other factors cannot be excluded altogether and need to be investigated.

Studies from the Indo-Pak Subcontinent show slightly different presenting features from what we found in our study, particularly in terms of increased bleeding tendency [19,20]. None of our patients had bleeding manifestations. The reason could be that most studies reported epidemics, during which a sizeable number of patients have the more serious form of infection, the DHF. On the contrary, our study gives the snapshot of the disease spectrum during a non-epidemic period. Additionally, it is possible that only patients with milder symptoms reported to our study centre, while the more seriously ill with hemorrhagic manifestations went to a hospital. In this regard it is pertinent to note that a recent study found that the adult population of Karachi had some knowledge about DF[21], and this might have caused the parents/guardian to seek treatment for the sick child from a hospital in case of bleeding manifestations. A blanching rash is a feature of DF

that was not seen in our patients presumably because patients were seen early in the course of the disease, whereas the rash classically develops after 3-4 days of onset of fever [22]. Additionally, rash may not be present in some patients with DF, mainly infants and young children [17]. The other signs and symptoms noted were similar to what has been previously reported from Pakistan, where most of the children presented with abdominal pain, vomiting, diarrhoea, cough or headache, resembling typhoid fever [4]. This makes the differential diagnosis problematic, especially in an area such as ours, where we have previously demonstrated a high incidence of typhoid fever [9]. The situation is compounded because of poor availability and/or affordability of blood tests for DEN, a fact that has kept the disease smoldering for quite some time while health authorities remained unaware of the situation.

A number of mathematical models have been developed to account for the seasonality of the disease and the vector. These incorporate factors such as rain, temperature, humidity, type of land, mosquito density, bite rate, life span, *etc*. [23, 24]. However, no clear evidence of cyclical or seasonal occurrence of DF emerged in our study; the disease appeared to occur independent of rainfall and temperature. One reason could be that yet other factors play roles in this relationship, *e.g*., infrastructure improvements being done during the study period in that area [24-26]. Secondly, the duration of our study was not long enough to comment firmly about this, however, the fact that supports our contention is that despite rains in July 2001, no increase in DF was observed for the next couple of months. In contrast, during the period of high dengue incidence, Karachi received almost no rain (Figure 3).

Figure 3. Relationship of dengue fever cases to weather conditions (Source of weather information: Mr. Abdul Qayoom, Computerized Data Processing Center, Meteorology Department, Government of Pakistan, Karachi, Personal Communication)
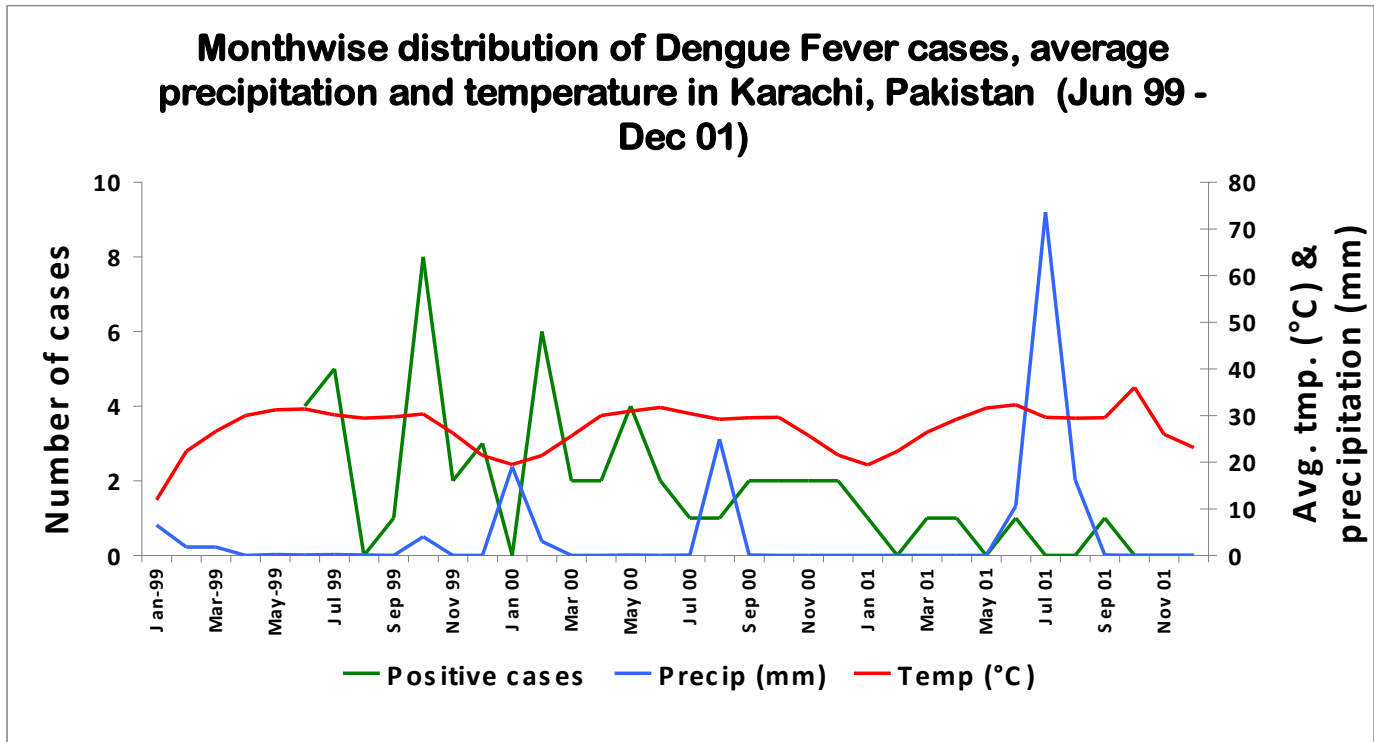
Conclusion

Is dengue fever endemic in children in slums of Karachi, Pakistan? Probably yes. The study suggests that DF is likely to be a significant problem in Karachi even during non-epidemic periods. Older children appear to be affected more as compared to younger ones. Disease presents itself mimicking typhoid fever so a high degree of suspicion is required to diagnose DF, especially during non-epidemic periods.

Further prospectively designed, community-based studies are needed to validate these findings. Until that time, DF may be included in the differential diagnosis of febrile illnesses without a definite focus of infection in children in Karachi slums.

Ethics Committee Approval
The study was approved by the Ethical Review Committee of the Aga Khan University, Karachi, Pakistan.

Acknowledgments

References
1. Kyle JL, Harris E (2008) Global spread and persistence of dengue. Annu Rev Microbiol 62: 71-92.
2. WHO (1999) Strengthening implementation of the global strategy for dengue fever/dengue haemorrhagic fever prevention and control. Report of the Informal Consultation, 18-20 October.
3. Chan YC, Salahuddin NI, Khan J, Tan HC, Seah CL, Li J, *et al*. (1995) Dengue haemorrhagic fever outbreak in Karachi, Pakistan. Trans R Soc Trop Med Hyg 89: 619-620.
4. Paul RE, Patel AY, Mirza S, Fisher-Hoch SP, Luby SP (1998) Expansion of epidemic dengue viral infections to Pakistan. Int J Infect Dis 2: 197-201.
5. Qureshi JA, Notta NJ, Salahuddin N, Zaman V, Khan JA (1997) An epidemic of Dengue fever in Karachi--associated clinical manifestations. J Pak Med Assoc 47: 178-181.
6. Jamil B, Hasan R, Zafar A, Bewley K, Chamberlain J, Mioulet V, *et al.* (2007) Dengue virus serotype 3, Karachi, Pakistan. Emerg Infect Dis 13: 182-183.

7. Khan E, Hasan R, Mehraj V, Nasir A, Siddiqui J, Hewson R (2008) Co-circulations of two genotypes of dengue virus in 2006 out-break of dengue hemorrhagic fever in Karachi, Pakistan. J Clin Virol 43: 176-179.

8. Khan E, Siddiqui J, Shakoor S, Mehraj V, Jamil B, Hasan R (2007) Dengue outbreak in Karachi, Pakistan, 2006: experience at a tertiary care center. Trans R Soc Trop Med Hyg 101: 1114-1119.

9. Siddiqui FJ, Rabbani F, Hasan R, Nizami SQ, Bhutta ZA (2006) Typhoid fever in children: some epidemiological considerations from Karachi, Pakistan. Int J Infect Dis 10: 215-222.

10. Ahmed S, Arif F, Yahya Y, Rehman A, Abbas K, Ashraf S, *et al*. (2008) Dengue fever outbreak in Karachi 2006--a study of profile and outcome of children under 15 years of age. J Pak Med Assoc 58: 4-8.

11. Wasay M, Channa R, Jumani M, Zafar A (2008) Changing patterns and outcome of Dengue infection; report from a tertiary care hospital in Pakistan. J Pak Med Assoc 58: 488-489.

12. Phuong HL, de Vries PJ, Nga TT, Giao PT, Hung le Q, Binh TQ, et *al*. (2006) Dengue as a cause of acute undifferentiated fever in Vietnam. BMC Infect Dis 6: 123.

13. Akram DS, Igarashi A, Takasu T (1998) Dengue virus infection among children with undifferentiated fever in Karachi. Indian J Pediatr 65: 735-740.

14. Crump JA, Youssef FG, Luby SP, Wasfy MO, Rangel JM, Taalat M, *et al*. (2003) Estimating the incidence of typhoid fever and other febrile illnesses in developing countries. Emerg Infect Dis 9: 539-544.

15. Butt N, Abbassi A, Munir SM, Ahmad SM, Sheikh QH (2008) Haematological and biochemical indicators for the early diagnosis of dengue viral infection. J Coll Physicians Surg Pak 18: 282-285.

16. Sudjana P, Jusuf H (1998) Concurrent dengue hemorrhagic fever and typhoid fever infection in adult: case report. Southeast Asian J Trop Med Public Health 29: 370-372.

17. Guzman MG, Kouri G (2002) Dengue: an update. Lancet Infect Dis 2: 33-42.

18. Kabilan L, Velayutham T, Sundaram B, Tewari SC, Natarajan A, Rathnasamy R, *et al*. (2004) Field and laboratory-based active dengue surveillance in Chennai, Tamil Nadu, India: observations before and during the 2001 dengue epidemic. Am J Infect Control 32: 391-396.

19. Narayanan M, Aravind MA, Thilothammal N, Prema R, Sargunam CS, Ramamurty N (2002) Dengue fever epidemic in Chennai--a study of clinical profile and outcome. Indian Pediatr 39: 1027-1033.

20. Ali N, Usman M, Syed N, Khurshid M (2007) Haemorrhagic manifestations and utility of haematological parameters in dengue fever: a tertiary care centre experience at Karachi. Scand J Infect Dis 39: 1025-1028.

21. Itrat A, Khan A, Javaid S, Kamal M, Khan H, Javed S, *et al*. (2008) Knowledge, awareness and practices regarding dengue fever among the adult population of dengue hit cosmopolitan. PLoS ONE 3: e2620.

22. Gubler DJ (1998) Dengue and dengue hemorrhagic fever. Clin Microbiol Rev 11: 480-496.

23. Nakhapakorn K, Tripathi NK (2005) An information value based analysis of physical and climatic factors affecting dengue fever and dengue haemorrhagic fever incidence. Int J Health Geogr 4: 13.

24. Bartley LM, Donnelly CA, Garnett GP (2002) The seasonal pattern of dengue in endemic areas: mathematical models of mechanisms. Trans R Soc Trop Med Hyg 96: 387-397.

25. Hossain MA, Khatun M, Arjumand F, Nisaluk A, Breiman RF (2003) Serologic evidence of dengue infection before onset of epidemic, Bangladesh. Emerg Infect Dis 9: 1411-1114.

26. Teixeira Mda G, Costa Mda C, Barreto ML, Mota E (2005) Dengue and dengue hemorrhagic fever epidemics in Brazil: what research is needed based on trends, surveillance, and control experiences? Cad Saude Publica 21: 1307-1315.

Corresponding Author

Zulfiqar Ahmed Bhutta, Department of Paediatrics, The Aga Khan University, PO Box 3500, Stadium Road, Karachi – Pakistan
Fax: (9221) 4934294; 4932095
E-mail: zulfiqar.bhutta@aku.edu