

Serodiagnosis of dengue infection using rapid immunochromatography test in patients with probable dengue infection

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Abstract

Objective: To determine the frequency of seropositive dengue infection using rapid immunochromatographic assay in patients with probable dengue infection as per WHO criteria.

Method: A cross-sectional observational study was conducted at Abbasi Shaheed Hospital, Karachi from July 2008 to January 2009. Patients presenting with acute febrile illness, rashes, bleeding tendencies, leucopenia and or thrombocytopenia were evaluated according to WHO criteria for probable dengue infection. Acute phase sera were collected after 5 days of the onset of fever as per WHO criteria. Serology was performed using rapid immunochromatographic (ICT) assay with differential detection of IgM and IgG. A primary dengue infection was defined by a positive IgM band and a negative IgG band whereas secondary infection was defined by a positive IgG band with or without positive IgM band.

Result: Among 599 patients who met the WHO criteria for dengue infection, 251(41.9%) were found to be ICT reactive among whom 42 (16.73%) had primary infection. Secondary infection was reported in 209 (83.26%). Acute phase sera of 348 (58.09%) were ICT non reactive. Four patients died because of dengue shock syndrome among which three had secondary infection.

Conclusion: Early identification of secondary infection in acute phase sera using rapid ICT is valuable in terms of disease progression and mortality. However in highly suspected cases of dengue infection clinical management should not rely on negative serological results (JPMA 60:936; 2010).

Introduction

Dengue virus infection has emerged as a major public health concern across the globe in terms of mortality, morbidity and public health cost. According to the WHO estimates¹ approximately 50 million dengue fever (DF) occurs worldwide every year and around 500, 000 cases of dengue haemorrhagic fever (DHF) require hospitalization each year with the mortality of 2.5 %. Dengue infection is the most prevalent mosquito borne Arbovirus infection in tropical and sub-tropical regions of the world and has been reported as endemic in more than 100 countries in Africa, America, eastern Mediterranean, south East Asia and western pacific.² The principle vector is a day-biting domestic mosquito *Aedes aegypti*; although a second dengue vector *Aedes albopictus* has been shown to be responsible for transmission of dengue in Asia.³

In Karachi the first confirmed epidemic of dengue infection was reported in 1994⁴ followed by another confirmed outbreak in 2005.⁵ The largest out break with maximum mortality occurred in 2006.⁶ Four antigenically different serotypes of dengue virus (DEN 1-to-DEN 4) have been identified. In Karachi during 1994 outbreak,⁴ DEN 1 and DEN 2 were isolated, where as in 2005 out break DEN 3 was detected.⁵ Co circulation of DEN 2 and DEN3 had been identified during 2006 outbreak in Karachi.⁷

Abbasi Shaheed hospital is among five major hospitals in public and private sector which provides tertiary care facilities in Karachi. For the past few years serological diagnosis of dengue infection has been performed by rapid ICT assay. Present study was designed to determine the frequency of ICT seropositive cases and to evaluate the role of rapid ICT test as a diagnostic aid in patients with suspected dengue infection. In dengue endemic areas like Karachi, serological evidence is useful to provide information on epidemiology of the disease which is essential to plan necessary measures to control and prevent dengue infection.

Patients and Methods

The present study is a hospital based cross sectional observational study conducted at Abbasi Shaheed Hospital, Karachi from July 2008 to January 2009. All patients of both genders and ages 13 years and above presenting with acute febrile illness, bleeding tendencies (petechiae or ecchymosis, epistaxis, gum bleeding, haematemeses or malena), leucopenia and or thrombocytopenia were included. Patients with haematological malignancies, bleeding diathesis, cirrhosis, enteric fever and malaria were excluded.

Patients were enrolled as probable cases of dengue fever as per WHO criteria of probable DF i.e. acute febrile illness with two or more of the following manifestations:

headache, retro-orbital pain, myalgia, arthralgia, rash, haemorrhagic manifestations, leucopenia and supportive serology, a positive IgM antibody test on serum samples collected five or more days after the onset of fever, or occurrence at the same location and time as other confirmed cases of dengue fever. DHF was defined as probable cases of dengue and haemorrhagic tendencies, thrombocytopenia and evidence of plasma leakage i-e > 20% rise in haematocrit or signs of plasma leakage (pleural effusion, ascites, or hypoproteinaemia). Platelets were transfused prophylactically in patients with platelet count < 20,000 or with bleeding manifestations.

Peripheral thick and thin smear for malarial parasite was performed in all patients, whereas blood culture was done in selected cases. Acute phase sera of all enrolled patients were collected after 5 days of onset of fever as per WHO criteria for serological diagnosis using rapid ICT assay with differential detection of IgM and IgG antibodies. The results were graded as reactive (visible band) and non reactive (no band). Repeat ICT was performed after 3 to 5 days of first sera (within 12 days of onset of fever) in non reactive cases. We did not collect the convalescent phase sera which is collected 7-21 days after the first sample. The rapid ICT is a qualitative membrane based immunoassay for the detection of dengue specific antibodies in whole blood, serum or plasma. This device consists of two components an IgG and IgM component, coated with ligand anti-human IgG and anti human IgM respectively. During testing, the specimen reacts with dengue antigen coated particles (type 1-4) in the test strip. The mixture then migrates upwards on the membrane chromatographically by capillary actions and is captured by ligand anti-IgG or anti-IgM, forming a visible band in specific region. The IgG cutoff in the test has been set to detect the high IgG levels characteristic of secondary infection, therefore the primary dengue infection was defined by a visible IgM band without a visible IgG band whereas secondary infection was defined by a positive IgG band with or without positive IgM band. Data was analyzed by SPSS version 10. Relevant descriptive statistics like, frequency, mean and percentages were calculated for presentation of data.

Results

Among 612 patients admitted with suspected dengue, 599 patients met the WHO criteria of probable dengue infection. Maximum number of cases were admitted in September (149), October (127), and November (139). Of total 599 patients, acute phase serum samples of 251 (41.9%) were found to be ICT positive. Among these, acute phase sera of 193 (76.89%) patients were positive after five days of onset of fever. Whereas, on repeat serology after 3-5 days (within 12 days of onset of fever), 58 (23.1%) collected sera were found to be ICT positive. Primary infection was reported in

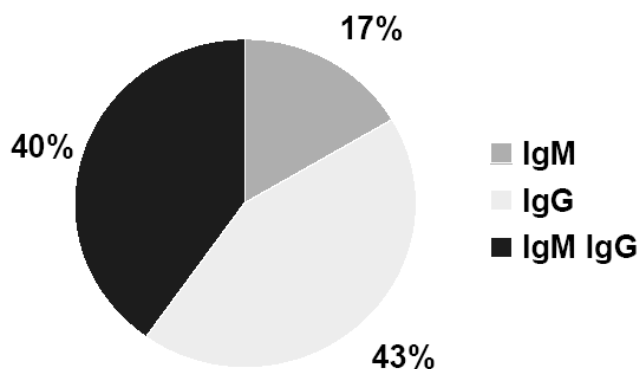


Figure: Percentage of patients with primary and secondary infection.

Table: Clinical and Laboratory Features of Patients with Probable Dengue Infection.

Clinical Features	ICT Positive n=251	ICT Negative n=348
Age (Years) 13-33	199 (79.28%)	266 (76.43%)
34-53	47 (18.72%)	71 (20.4%)
>53	5 (1.99%)	11 (3.16%)
Fever	251 (100%)	348 (100%)
Myalgias	158 (62.94%)	226 (64.9%)
Arthralgias	51 (20.3%)	77 (22.12%)
Headache	169 (67.3%)	233 (66.95%)
Abdominal Pain	125 (50%)	161 (46.2%)
Rash	178 (70.9%)	240 (69%)
Mucosal Bleeding	130 (51.7%)	170 (48.8%)
Leucopenia <4000/mm ³	120 (47.8)	173 (49.71%)
Platelet count <50, 000 mm ³	175 (69.72%)	172 (49.72%)
50, 000 – 100, 000 mm ³	78 (31.0%)	111 (32.47)
>100, 001 but <150, 000	8 (3.81%)	0

42 (16.73%) patients. Secondary infection (IgG with or without IgM) was reported in 209 (83.26%) patients (Figure). One hundred and sixty two (64.5%) ICT reactive patients were males and 89 (35.45%) were females making the ratio of M: F = 1.8: 1. The maximum number of patients presented in the 13-33 years age group 199 (79%) followed by 34- 53 years, 47 (18.7%) (Table).

Of total 251 ICT reactive patients, 132 (52.58%) required platelet transfusion due to bleeding tendencies or platelet count < 20,000. Eleven (26.19%) patients with primary infection whereas 121 (57.89%) with secondary infection were transfused. Three hundred and forty eight (58.09%) patients with highly suspected dengue infection were found to be ICT non reactive. Two hundred and thirty seven (68.1%) were males and 111 were females (M; F 2:1). Maximum number of patients presented in the age group of 13-33 years. Of these non reactive cases, 176 (50.58%) required platelet transfusions. Details of clinical and laboratory manifestations of ICT reactive and non reactive cases of probable dengue infection are given in Table.

Four patients died with dengue shock syndrome among whom three had secondary infection (two were IgM + IgG positive) and one patient had primary infection. Case fatality rate was 0.7%.

Discussion

In Karachi cyclic epidemic pattern of dengue infection has been observed in post monsoon season. Relative increased humidity of post monsoon season has been shown to be the contributing factor for increased dengue propagation in *A.aegypti*.⁸ During 2006 maximum number of cases were reported from August to October.⁶ Our study also reports the peak incidence of dengue infection in the months of August to November, highlighting the months suitable for dengue transmission and need of effective pesticide spraying after rain fall. Demographic characteristics like age distribution and gender differences are important for the successful planning of public health programmes and effective control of communicable diseases.

Differences in infection rates and severity of disease among males and females are reported in few hospital based studies from other countries. Studies from South America report almost equal male to female ratio,^{9,10} whereas studies from India and Bangladesh showed predominance of males.^{11,12} Present study also found nearly twice the number of male patients compared to female in clinically diagnosed dengue fever (both ICT reactive and ICT non reactive). The lower infection rates in females of Asian community might be attributed to lower reporting rate and the fact that they remained stationed at home and are less exposed to this vector born infection.

Some studies from Asia revealed higher case fatality rate (CFR) among females despite high infection rate in males.^{11,13} In contrast, of four deaths in our study sample two were females. Children and adolescence have been reported to be the predominant group in Southeast Asia including some parts of India.^{14,15} In the present study data of both ICT reactive and non reactive cases of dengue fever revealed pre dominant involvement of young adults in the 13-33 years age group. This finding is consistent with another local study from Karachi¹⁶ and studies from other endemic countries.^{17,18} A hospital based study from Bangladesh reported highest percentage of cases in the 18-33 years age group. The increasing incidence and CFR of dengue infection among young adults merit special consideration during control and prevention of public health programme. Of significance are all four case fatalities in the present study which occurred in the age group of 13 -33 years.

Laboratory evidence is required for epidemiological surveillance of any endemic disease. Virus isolation, molecular diagnosis using reverse transcriptase polymerase chain reaction (PCR) and serological methods have been used

for laboratory confirmation of dengue infection. Viral isolation takes several days and is not available in many endemic countries. Although PCR is a useful tool for identification and dengue strain characterization, its widespread use for diagnosis of dengue infection is limited due to high cost, especially in developing countries.

In the clinical setting diagnosis of dengue infection is primarily based on serology by detecting dengue specific antibodies. Serological tests that have been available for the detection of dengue specific antibodies include haemagglutination inhibition (HAI) assay, enzyme linked immunosorbent assay (ELISA), dot blot assay, dip stick and rapid ICT assay. To distinguish between primary and secondary dengue infection HAI, capture IgM and IgG ELISA, and rapid ICT have been employed.

The rapid ICT is a commercial kit that incorporates 4 recombinant proteins from DEN 1- to- DEN 4. This assay detects both IgM and IgG antibodies in 15 minutes and helps in differentiation of primary and secondary dengue infections.

Different immunological response patterns have been observed during primary and secondary dengue infections. In primary infection specific IgM levels develop 5-6 days after the onset of illness and IgG levels after 7-10 days and persist for life at a lower titer (HAI 1:640). During secondary infection high titer of IgG (HAI 1:2,560) appears earlier. IgM antibodies are either present at lower titer or absent during secondary infection.¹⁹ In present study using ICT assay acute phase sera of 108(51.68%) cases among 209 secondary infections were reactive for IgG without IgM.

For primary dengue infection, a capture IgM ELISA showed that 80% of sera exhibited detectable levels of IgM antibody by fifth day of febrile illness and 99% of sera tested IgM by 10th day.²⁰ In our study acute phase sera of 251(41.9%) cases were found to be ICT reactive, among whom 76.89% were reactive after five days of fever. Twenty three percent sera turned out to be ICT reactive on repeat serology after 3-5 days. We therefore suggest repeat serology in highly suspected cases of dengue infection. In a study conducted by Sang et al.²¹ 57% of dengue cases were detected with acute phase sera whereas sensitivity rose to 99% when both acute and convalescent- phase sera were analyzed. In our study as no convalescent- phase sera were tested, which usually requires collection of sera after hospital discharge, therefore, the percentage of ICT reactive cases might have been higher if paired sera were analyzed. In contrast to our findings Cuzzubbo et al²² reported that 89% of dengue infections were diagnosed using acute phase sera only. We recommend further evaluation of rapid ICT assay using paired sera and comparison with other assay like capture ELISA.

Detailed clinical and laboratory evaluation of all 599 patients (both ICT reactive and non reactive) was consistent with dengue infection. Based on these findings and clinical outcome presumptive diagnosis of dengue infection was maintained in all ICT non reactive cases. Among ICT non reactive cases significant percentage (50.58%) required platelet transfusion. Our study highlights the fact that patients with high degree of clinical suspicion of dengue infection require careful monitoring and clinical management should not rely on negative serological results.

Secondary infection has been reported to be more prevalent in dengue endemic areas.²⁵ Present study also reports that significant percentage of patients had secondary infection (83.26% Vs 16.73%) which can be explained by sequential circulation of different dengue serotypes during multiple outbreaks of dengue infection in Karachi. Prior sensitization by a heterologous dengue serotype increases the relative risk of acquiring more severe disease in secondary infection than in primary infection. In the present study significantly greater percentage of patients with secondary infection (57.89%) required platelets transfusion as compared to primary infection (26.19%) moreover three out of four patients who died from dengue shock syndrome had secondary dengue infection.

Conclusion

Identification of secondary infection early during an acute phase of illness is valuable for the clinician due to higher risk of progression to life threatening dengue haemorrhagic fever and dengue shock syndrome and therefore of utmost importance to reduce the case fatality rate.

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References

- World Health Organization. Dengue and dengue hemorrhagic fever. Fact sheet No.117, 2002.
- World Health Organization. Dengue/dengue hemorrhagic fever. Weekly Epidemiological Report 75, 2000; 24:193-200.
- Shandera W, Koo H. Infectious disease: Viral and Rickettsial. In Mcphee JS, Papa dakis MA, Tierney Jr LM, eds. Current Medical Diagnosis and Treatment. 46th ed. New York: Mc GrawHill 2007; pp 1407-8.
- Chan YC, Salahuddin NI, Khan J, Tan HC, Seah CL, Li J, et al. Dengue haemorrhagic fever outbreak in Karachi, Pakistan, 1994. *Trans R Soc Trop Med Hyg* 1995; 89: 619-20
- Jamil B, Hasan R, Zafar A, Bewley K, Chamberlain J, Mioulet V, et al. Dengue virus serotype 3, Karachi, Pakistan. *Emerg Infect Dis* 2007; 13: 182-3.
- Khan E, Siddique J, Shakoor S, Mehaj V, Jamil B, Hassan R. Dengue outbreak in Karachi Pakistan, 2006: experience at tertiary care center. *Trans R Soc Trop Med Hyg* 2007; 101: 1114-9.
- Khan E, Hasan R, Mehraj V, Nasir A, Siddique J, Hewson R. Co-circulations of two genotypes of dengue virus in 2006 out-break of dengue hemorrhagic fever in Karachi, Pakistan. *J Clin Virol* 2008; 43: 176-9.
- Thu HM, Aye KM, Them S, The effect of Temperature and humidity on dengue virus propagation in *Aedes aegypti* mosquitos. *South East Asian J Trop Med Public Health* 1998; 29: 280-4.
- Rigau-Perez JG, Aayala-Lopez A, Vorndam AV, Clark GG: Dengue activity in Puerto Rico during an interepidemic period (1995-1997). *Am J Trop Med Hyg* 2001; 64: 75-8.
- Vasconcelos PF, Travassos da Rosa ES, Travassos da Rosa JF, de Freitas RB, Degallier N, Rodrigues SG, Travassos da Rosa AP: [Outbreak of classical fever of dengue caused by serotype 2 in Araguaiana, Tocantins, Brazil]. *Rev Inst Med Trop Sao Paulo* 1993; 35: 141-8.
- Agarwal R, Kapoor S, Nagar R, Misra A, Tandon R, Mathur A, Misra AK, Srivastava KL, Chaturvedi UC: A clinical study of the patients with dengue hemorrhagic fever during the epidemic of 1996 at Lucknow, India. *Southeast Asian J Trop Med Public Health* 1999; 30: 735-40.
- Ynus E: National guidelines for clinical management of Dengue and Dengue Haemorrhagic Fever. Dhaka, Disease Control Directorate, Directorate General of Health Services; 2000.
- Kabra SK, Jain Y, Pandey RM, Madhulika, Singhal T, Tripathi P, Broor S, Seth P, Seth V: Dengue haemorrhagic fever in children in the 1996 Delhi epidemic. *Trans R Soc Trop Med Hyg* 1999; 93: 294-8.
- Monath TP:Dengue:The risk to develop and developing countries.*Proc Natl Acad Sci USA* 1994; 91: 2395-400.
- Abhyankar AV,Dash PK,sexena P,Bhargava R,Parada MM,Jana AM. Comparison of a dipstick Dot-ELISA with commercial assay for anti-dengue virus IgM antibodies.*Viral Immunology* 2006; 19: 630-6.
- Riaz MM,Hilal H,Mumtaz K,Khan MS,Patel J,Tariq M et al.out break of dengue fever in Karachi 2006: a clinical perspective 2009; 59: 339.
- Rahman M, Rahman K, Siddique AK, Shoma S, Kamal AH, Ali KS, Nisalak A, Breiman RF: First outbreak of dengue hemorrhagic Fever, bangladesh. *Emerg Infect Dis* 2002; 8: 738-40.
- Low JGH,Ooi EE,Tolfvenstam T Lew YS,Hibberd ML,Ng LC,et al.Early dengue infection and outcome study(EDEN) -study design preliminary findings.*Ann Acad Med Singapore* 2006; 35: 783-9.
- Wilder-Smith A, Schwartz E. Dengue in travelers. *N Engl J Med* 2005; 353: 924-32.
- Dengue haemorrhagic fever diagnosis,treatment,prevention and control.2nd edition Geneva:World Health Organization 1997.
- Sang CT, Hoon LS, Cuzzubbo A, Devine P. Clinical evaluation of rapid immunochromatographic test for the diagnosis of dengue infection. *Clin Diagn Lab Immunol* 1998; 5: 407-9.
- Cuzzubbo A J, Endy TP, Nisalak A, Kalayanarooj S, Vaughn DW, Ogata S A, et al. Use of Recombinant Envelope Proteins for Serological Diagnosis of Dengue Virus Infection in an Immunochromatographic Assay. *Clin Diagn Lab Immunol* 2001; 8: 1150-55.
- Vaughn DW, Green S, Kalayanarooj S, Innis BL, Nimmannitya S, Suntayakorn S, Rothman AL, Ennis FA, Nisalak A. Dengue in the early febrile phase: viremia and antibody responses. *J Infect Dis* 1997; 176: 322-30.