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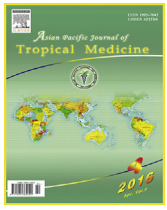

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Dengue outbreak in Swat and Mansehra, Pakistan 2013: An epidemiological and diagnostic perspective

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ABSTRACT

Objective: To high light some epidemiological, clinical and diagnostic features of dengue fever during an outbreak and the role of different diagnostic techniques to achieve the highest level of accuracy in results.

Methods: Blood samples (n = 323) were collected along with epidemiological and clinical data from suspected dengue patients who visited different hospitals in Swat and Mansehra district of Pakistan between May–November 2013 during a dengue outbreak. Samples were tested for the detection of viral nucleic acid by real-time PCR, non structural protein-1 (NS1) antigen and IgM antibodies by ELISA.

Results: Out of 323 cases with clinical dengue infection, 304 were positive by one or more diagnostic parameter; 201 samples were positive by real-time PCR, 209 were positive by NS1 ELISA and 190 were positive by IgM antibodies. Sensitivities of real-time PCR and NS1 ELISA were comparable for early diagnosis of dengue virus infection, IgM antibody detection assay was found useful for the diagnosis in the samples collected later than day 5 of onset.

Conclusions: The use of real-time PCR or detection of non structural protein NS1 by ELISA followed by IgM antibodies detection can be recommended for early diagnosis of dengue virus infection with a high level of accuracy.

1. Introduction

Dengue fever is an important emerging and re-emerging arboviral infection and major public health problem of tropical and subtropical regions of the world [1,2]. According to the World Health Organization 2.5 billion people and 124 countries are at risk of dengue infection with over 100 million cases of dengue virus (DENV) infection and 30 000 estimated deaths. In most of the dengue endemic countries the DENV has caused regular cyclic epidemics after every 3–5 years [3].

Dengue fever is caused by the DENV which belongs to the genus *Flavivirus*, family flaviviridae and has been classified into four (DENV-1-4) serotypes and ten genotypes on the basis of nucleotides differences in the sequence of envelope gene [4,5]. The DENV infection is classified into three categories ranging from mild dengue fever to severe life threatening dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) [6]. There are 500 000 cases of DHF and DSS reported annually which are related to severe disease manifestation characteristic of secondary infection [7,8].

Pakistan has a temperate climate and the DENV has been endemic for many years [9–12]. However, since 2006 dengue outbreaks have been reported every year and co-circulation of multiple dengue serotypes has been reported [9]. The presence of rich fauna, vast agricultural land, open irrigation channels, artificial water reservoirs for power generation and floods from heavy rainfall provide ample breeding sites for the mosquito vector(s). The DENV vector activities differ according to season in different geographical areas of Pakistan and typically the incidence of cases increases after the rainy season [7].

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Various diagnostic methods are currently available for the diagnosis of DENV infection, like virus isolation, conventional reverse transcriptase polymerase chain reaction (RT-PCR), real-time PCR, genomic sequencing, ELISA for detection of viral antigen and IgM, IgG antibodies [13]. Virus isolation and molecular techniques are highly sensitive assays but often need a specialized laboratory setting and highly trained staff as well. In a developing country like Pakistan, serology is the more commonly used method for diagnosis of dengue infections. Although RT-PCR and viral antigen non structural protein-1 (NS1) detection by ELISA are highly sensitive techniques for the early diagnosis of DENV, they are not routinely used in public health laboratories due to resource limitations.

The aim of the present study was to evaluate the diagnostic accuracy of different diagnostic tests such as NS1 ELISA, real-time PCR and IgM ELISA for the detection of DENV infection during an outbreak and correlate with clinical data especially the date of onset of infection.

2. Materials and methods

A total of 323 blood specimens collected from the suspected DENV cases were transported to the Department of Virology National Institute of Health Islamabad maintaining cold chain. This study was approved by the Internal Review Board of National Institute of Health, Islamabad, Pakistan.

Sera were separated by centrifuging the whole blood at 3000 rpm for 10 min. Each sample was analyzed for the detection of NS1 antigen and IgM antibodies by ELISA and viral RNA by real-time PCR. All serum samples were stored at −80 °C until further use.

2.1. Detection of NS1 antigen

NS1 antigen was detected by using Platelia dengue antigen detection kit (Biorad Laboratories, Marnes-la-Coquette, France) according to the instruction provided by the manufacturer. Briefly, sample diluents buffer (50 μL) was added to each well of microplate followed by the addition of serum, positive and negative controls (50 μL) and conjugate (100 μL). After the addition of conjugate, microwell plate was covered with an adhesive plate sealer and incubation at 37 °C for 90 min. After incubation, plate was washed and 160 μL of substrate was added followed by incubated at room temperature for 30 min. The presence of NS1 antigen was demonstrated by a color development and reading of optical density (OD) at a wavelength of 450 nm. Test results were determined by comparing the OD values of sample to the OD values of cut-off controls.

2.2. Viral RNA detection by real-time PCR

RNA was extracted using 140 μL of serum samples with QIAamp Viral RNA Mini Kit (Qiagen, Germany) according to the protocol provided by manufacturer.

Serotype specific four-plex, real-time TaqMan RT-PCR was carried out according to the CDC protocol developed by Johnson *et al* [14]. Briefly, four-plex reaction mixtures of 25 μL were run for each DENV serotype (DENV-1-4) in ABI7500 real-time thermocycler using the SuperScript III Platinum one-step qRT-PCR kit (Invitrogen). Amplifications for each serotype was carried out separately in 25 μL reaction mixture containing 5 μL extracted RNA, 12.5 μL of 2 × reaction mixture, 0.5 μL enzyme mix, 2 μM of each primer and 1 μM of TaqMan probe. The cycling conditions were as follows: RT step 50 °C for 10 min, initial denaturation at 95 °C for 5 min and 45 cycles at 95 °C for 15 s and at 60 °C for 60 s. The data was analyzed using software SDS version 1.4.

2.3. Detection of IgM antibodies

DENV specific IgM antibodies were detected using DENV IgM Capture ELISA (Panbio, Queensland, Australia) according to the protocol provided by the manufacturer. Results were interpreted by the calculation of cut-off and index values.

2.4. Statistical analysis

Statistical analysis of data was performed by chi-square test and student *t*-test using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). A P value of <0.05 was considered as significant.

3. Results

A total of 323 serum samples were collected from suspected dengue fever cases during the month of May–November 2013 (Table 1). Gender distribution was significant (P = 0.006) with 65% male. Age was found to be a significant characteristic among dengue positive and dengue negative cases by independent sample *t*-test (P = 0.025). Most effected 49.5% (160/323) age group was 16–30 years old. Mean days after illness was 4.78 ± 2.06 for dengue positive cases which was found significant compared to dengue negative group (P = 0.001). Platelets count and TLC values were significantly different between dengue positive and negative groups.

3.1. Month wise distribution of cases

During the study period from May–November 2013 the highest number of dengue fever cases was reported in September (Figure 1).

3.2. Symptoms correlation

The frequency of clinical symptoms was unequal in patients with acute dengue infection compared to dengue negative cases. Fever, body ache, retro-orbital pain, rash and bleeding were the most common symptoms among dengue positive cases (P > 0.05) (Table 2).

Table 1
Epidemiological characteristics of patients enrolled in the study (n = 323).

Clinical and demographic features	Dengue positive	Dengue negative	*P*-value
Number of patients [*n* (%)]	304 (94)	19 (6)	–
Mean age (years) (mean ± SD)	29.35 ± 19.44	34.11 ± 15.32	0.025
Male [*n* (%)]	198 (61)	14 (7)	0.006
Female [*n* (%)]	106 (33)	5 (5)	
Days after illness onset (mean ± SD)	4.78 ± 2.06	6.35 ± 3.48	0.001
Platelets counts (cell/μL)	4344	2418	0.001
TLC count (cell/μL)	137	948	0.001