COMPARISON OF SINGLE-TUBE MULTIPLEX REVERSE TRANSCRIPTASE PCR WITH CLINICAL DATA IN THE DIAGNOSIS OF DENGUE FEVER

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Introduction

Dengue is one of the most important, life threatening tropical viral infection of humans in the world today. Around 100 million cases of classical dengue fever (DF) and up to half a million cases of dengue hemorrhagic fever (DHF) are reported annually (WHO, 1999). Dengue is transmitted to humans by the mosquito vector *Aedes aegypti*. There are 4 distinct virus serotypes, DEN-1, 2, 3 and 4, each capable of producing a wide spectrum of signs and symptoms (Kumaria and Chakravarti, 2005). The World Health Organization (WHO) has recognized and designated Sri Lanka as a dengue hyper endemic country of the Southeast Asian region (WHO, 1999), thus emphasizing the need for a confirmatory diagnostic test to be performed in the early phase of illness. Furthermore, identification of causative serotypes is crucial since DHF can develop due to sequential infection by distinct serotype (Harris *et al.*, 1988).

Single-step reverse transcriptase polymerase chain reaction (RT-PCR) offers a sensitive, specific and rapid diagnostic test for detection as well as serotypic characterization of dengue virus in clinical samples (Kumaria and Chakravarti, 2005; Harris *et al.*, 1988).

In the present study, we used a single-tube muliplex RT-PCR assay to detect and type dengue virus in clinical specimens. The results of the RT-PCR assay were compared with clinical data of the patients.

Methodology

Serum samples were obtained from 162 individuals with suspected dengue fever based on clinical aspects (as defined by the WHO) on the fifth day of fever. Sera were collected from Mawanella Hospital (n=82), Kandy General Hospital (n=65) and Padaviya Hospital (n=15).

At the time of sera collection, clinical data were recorded on a structured form. Clinical data included recording of presenting symptoms and their duration, vital parameters such as pulse and blood pressure, white blood cell (WBC) count, platelet count, packed cell volume (PCV) and haemoglobin (Hb). In addition, basic demographic data such as age, gender, residence and epidemiological data were recorded.

RNA was extracted from serum samples by using guanidine thiocyanate method and purified by using silica gel and eluted in 14 µl of RNase-free sterile deionized, distilled water. Reverse transcription and PCR

amplification was carried out according to the method described by Harris *et al* (1988) with slight modifications. A total of 5 µl of extracted RNA was used as a template in a 25 µl RT-PCR reaction volume. Reverse transcription reaction was carried out at 42^{0}C for 60 minutes. Optimized PCR reaction conditions were 94^{0}C for 30 seconds, 55^{0}C for 30 seconds, 72^{0}C for 1 minutes, 40 cycles followed by 72 ^{0}C for 7 minutes to complete the reaction.

Results and Discussion

Out of the 162 clinical samples tested for Dengue virus, 57 samples were positive (Table 1). Of the positive samples, all 57 were positive for the serotype DEN-2. This is consistent with the findings that DEN-2 is the main circulating dengue serotype in Sri Lanka (Kularatne *et al*., 2006).

The RT-PCR results were compared with the clinical data (Table 2), to find whether there was any correlation between the clinical picture of the patients with that of the outcome of the RT-PCR result. We observed that there is no statistically significant difference between platelet count, Hb%, PCV and WBC count on admission between PCR positive and negative groups.

Table 1. RT-PCR results of clinical sample

Hospital	RT-PCR Positive	RT-PCR Negative	Total
Mawanella	24	58	82
Kandy	28	37	65
Padaviya	05	10	15
Total	57	105	162

Table 2. Comparison of clinical data with RT-PCR results[a]

Clinical data	RT-PCR Positive	RT-PCR Negative
Platelet x10^9/L	121	114
PCV	43.6	40.1
Hb%	13.6	12.5
WBC x10^9/L	5.0	6.1

[a]Mean values are given

Furthermore, results for the presence of IgM and IgG antibodies (carried out in MRI) in the serum samples obtained from Mawanella Hospital were compared with the RT-PCR results. Of the total number of 82 samples, 41 samples (50%) gave a positive result in the antibody test, but gave a negative result in the PCR assay. Only 19 samples (23%) gave a positive result in both methods of testing while 15 (18%) were found to be negative in both methods of testing. There were 7 (8%) samples, that were PCR positive but giving a negative result in the antibody test. This clearly indicates that molecular diagnosis of dengue fever by RT-PCR is a better tool for detection of dengue fever at the early stages rather than the testing of antibody levels, since it yields a high percentage of both false positive and false negative results.

At present we have a problem of facing worldwide pandemic influenza viral infections such as, H1N1 and H5N1. Clinical presentation of pandemic influenza and dengue fever is almost same. In this context, rapid and sensitive method of diagnosis of Dengue fever is very important to prevent over diagnosis at the initial stage.

In conclusion, since at present there is no vaccine or specific cure available for dengue infection, it is important that the method of diagnosis is both sensitive and rapid. This will enable in bringing down the mortality rate as well as provide an early warning of dengue epidemics.

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