Prevalence of All Four Dengue Virus Serotypes Confirmed By Using Real Time RT-PCR among Population of Lahore Pakistan

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Abstract
Dengue fever is an emerging disease in Pakistan with uninterrupted increase in number of cases since 2006 Dengue fever epidemic in Lahore Pakistan. This study was planned to identify serotypes of dengue virus (DENV) involved in epidemic of 2008. The patients were screened in the microbiology laboratory with immunochromatographic kits then confirmed by antibody capture Enzyme linked immunosorbent assay (ELISA). Real Time Reverse Transcriptase-Polymerase Chain Reaction (Real Time RT-PCR) was performed by Real Time system to identify its serotype. Among analyzed 12 acute phase confirmed dengue patients, 50.74 % (n=7) were men and 49.26% (n=5) were women. Real Time Reverse Transcriptase-Polymerase Chain Reaction (Real Time RT-PCR) was performed by Real Time system to identify its serotype. Among analyzed 12 acute phase confirmed dengue patients, 58.33 % (n=7) were men and 41.66% (n=5) were women. On Real time RT-PCR, Dengue virus (DENV) was detected in 83.33 % (n=10) patients. Among them Serotype dengue-4 (DENV-4) was detected in 40% (n=4) patients, DENV-2 in 30% (n=3) patients, DENV-1 in 10% (n=1) and DENV-3 in 20% (n=2) patients. This reveals first time in Pakistan the presence of all four serotypes of dengue virus in Lahore, which desires vigilant monitoring by laboratory-based surveillance in order to foresee the nature of future epidemics.

Keywords: Dengue Virus (DENV), Serotypes, Epidemic, reverse transcriptase-polymerase chain reaction (RT-PCR).

Introduction
In the tropics and subtropics, Dengue fever (DF) is still the most common Arthropod-borne infection (1, 2). Dengue infection is one of the most rapidly expanding diseases known to mankind with an estimate of 50 million new cases worldwide annually. It is currently transmitted in more than 100 countries and the population at risk of infection ranges in billions. Dengue fever is classified as a major global health problem by World Health Organization (WHO). This may be due to rapid global dissemination of its vector. Moreover, there is a frequent incidence of dengue outbreaks with multiple serotypes and the presentation of the disease in its severe forms namely, Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) (2). Numerous theories were proposed to define the molecular basis of DHF. According to most of them, the development of DHF is basically determined by host immune factors in relation to previous exposure to multiple DENV serotypes (8-10). This theory is supported by the fact that infection with a specific type of dengue virus produces antibodies in the body which lack cross-reactivity, i.e., the primary infection with one serotype provides lifelong immunity to that particular serotype but is unable to confer immunity against other serotypes, thus will not prevent further infection with other DENV serotypes. These non-neutralizing cross-reactive antibodies could activate the Fc-receptor bearing immune cells through the phenomenon of Antibody-dependent enhancement of infection. This is the sequential infection hypothesis for the...
The development of DHF/DSS (10), which may secondarily cause immune activation by monocytes/macrophages/lymphocytes activation and complement activation with resultant cytokine production (11). In Pakistan as there is no evidence yet available about the prevalent serotypes therefore this study was initiated to know about the serotypes prevalence so that considering it a comprehensive disease control strategy will be formulated and promulgated.

Materials and Methods

From the patients, a total of 12 acute-phase samples were collected within 3 days after the onset of symptoms. In the lab, screening devices based on immunochromatography (ICT-Dengue) were used for detecting IgM antibodies against DENV. In the next step, all seropositive patients were subjected to ELISA method to confirm the seropositivity. Anti-human IgM capture ELISA (MAC-ELISA) was performed using Humareader Single (strip reader) and Original Multiskan EX (Plate reader) according to the manufacturer’s instructions. Patients chosen for RT-PCR were the ones who came to the hospital within 2-3 days of onset of high-grade fever. Serum was separated aseptically by centrifuging the blood sample at 3000-4000 rpm for 5-10 minutes. Real-time RT-PCR was performed to detect the specific DENV RNA and its different serotypes (5, 28). Virus RNA was extracted from serum using the QIAmp Viral RNA mini kit (Qiagen, Germany). Extracted RNA was carefully handled in order to avoid RNA degeneration and then stored at -70°C or used for RT-PCR immediately. The RT-PCR was performed with QIAmp ultra-sense RNA Mini-kit, by using SYBR green method, according to the manufacturer’s instructions. The amplification and detection was done using Artus Dengue RT-PCR kit on Real-time PCR system Rotorgene-3000. The amplified PCR products were sent to the “Centre for Applied Microbiology (CAMB) laboratory” which is a research microbiology laboratory. CAMB laboratory had confirmed the sequence analysis for each DENV serotype.

Results

Real Time Reverse Transcriptase-Polymerase Chain Reaction (Real Time RT-PCR) was performed by Real Time system to identify its serotype. Among analyzed 12 acute phase confirmed dengue patients, 58.33 % (n=7) were men and 41.66% (n=5) were women. On Real time RT-PCR, Dengue virus (DENV) was detected in 83.33 % (n=10) patients. Among them Serotype dengue-4 (DENV-4) was detected in 40% (n=4) patients, DENV-2 in 30% (n=3) patients, DENV-1 in 10% (n=1) and DENV-3 in 20% (n=2) patients.

Discussion

In the previous epidemics occurring in different regions of Pakistan, not all four serotypes of dengue virus had been identified simultaneously. Our observation has truly turned Pakistan from an endemic area for dengue infection to a hyperendemic area like many other Asian countries, e.g., India, Taiwan, Thailand, Malaysia, etc., (10, 12, 15,16). These multiple serotypes were found co-circulating during this epidemic as detected by real time RT-PCR. Previously, ELISA assay used for dengue virus serotype identification failed to detect multiple serotypes in acute viremia; therefore, the aforementioned method should be used as a tool for the rapid identification and serotyping of DENV.

Furthermore, we have observed that all dengue serotypes can produce severe dengue illness. There was a high percentage of DHF among patients in 2008 outbreak which can also be a sign of hyperendemicity of dengue virus in Punjab and its greater likelihood elsewhere in Pakistan. DHF/DSS is still a major cause of death in children in Southeast Asia (13). Certain demographic and societal changes are thought to be associated with the reappearance of lethal dengue infection over the past 50 years (17, 18). The factors responsible for such an enormous expansion are rapid population growth, peri-urbanization with inadequate public health systems, lack of vector control, climatic variability and rainfalls, and increased travel (especially air travel) to endemic areas (5). Due to aforementioned factors, there is an increase in the reportable cases of dengue infection in different countries with more severe forms of the disease (1, 2, 4). This has also led to a change in the distribution of dengue epidemic in several countries in American region where, before 1980s, either there were no dengue cases (non-endemic areas) or very few dengue cases (hypo-endemic areas). Later, in 1980s and 1990s, dengue infection occurred with multiple serotypes in those countries (now hyper-endemic areas) (1, 2). All of these factors must be addressed assertively to control the spread of dengue and other arthropod-borne infections.
This reveals first time in Pakistan the presence of all four serotypes of dengue virus in Lahore, which desires vigilant monitoring by laboratory-based surveillance in order to foresee the nature of future epidemics.

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**References**