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An introduction to dengue-disease diagnostics

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ABSTRACT

In this review, we discuss how dengue disease can be diagnosed accurately at the viremia phase and the fever phase. Diagnostic tools currently used to detect dengue virus (DENV) are virus isolation, capture IgM and IgG ELISA, real-time polymerase chain reaction (RT-PCR), and immunochromatography of the dengue NS1 antigen. First, we explain the advantages, the challenges and the limitations of different diagnostic tests. This account is followed by several examples using biosensors for detection of several important DENV biomarkers. Finally, we discuss our opinions regarding future perspectives in this field.

Contents

1. Introduction

Dengue viruses (DENV) are arboviruses (arthropod-borne viruses) and classified under the Flavivirus genus, which also contains the West Nile virus, yellow fever virus, and encephalitis virus. The dengue virus is enveloped, is spherical with a diameter of 50 nm and consists of a single, positive-sense RNA genome of about 11,000 nucleotides with only one open-reading frame. This open-reading frame encodes a single polyprotein precursor arranged in an NH_2-C-prM-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-COOH sequence. Co-translational and post-translational proteolytic processing gives rise to three structural proteins that form the virion: the C protein encapsulates the viral genomic RNA to form the nucleocapsid, and the nucleocapsid is enveloped by a lipid bilayer in which viral membrane (prM protein) and envelope protein (E protein) are embedded [1,2]. Dengue non-structural proteins (NS1–NS5), which are expressed in infected cells, are essential for virus replication, virion assembly, and avoiding the host immune response. Primarily, the non-structural proteins exist in the cytoplasm to form replication products that subsequently help in viral RNA synthesis. However,

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DENV NS1, a hydrophilic membrane-associated homodimer, is synthesized in the endoplasmic reticulum. The C-terminal residues of NS1 are probably involved in NS1-associated pathogenesis, as the mutation in NS1 protein is known to disrupt RNA synthesis, so exploration of the three-dimensional structures of the NS1 protein and the viral NS1-NS2A catalytic domain will contribute to understanding of the conformation of NS1 subunit, and its implication in viral pathogenesis. NS2B works as chaperone that participates in the folding of the NS3 subunit in its active conformation, and is implicated in regulating substrate-enzyme interaction and in membrane association. The best characterized DENV non-structural proteins are NS3 and NS5, which are multifunctional proteins involved in enzymatic activities. NS4 consists of two subunits, NS4A and NS4B; NS4A is involved in intracellular membrane modulation and its C-terminal end helps in the translocation of NS4B subunit. However, the function of the NS4B subunit is not understood, although recent studies suggest that it acts as an interferon antagonist [2,3].

DENV is an RNA Flavivirus that has four serotypes, numbered DENV1, DENV2, DENV3 and DENV4 and categorized by the number of antigens that the viruses have in common. Dengue was earlier classified via the World Health Organization categorization into dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Case evaluation has not always identified an infected person when they are in danger of harsh sickness, and the classification is simply as having ungenerous or severe infection. A patient who is infected with one serologic type does not acquire immunity to dengue's other serotypes. The primary infection of DF could convert to harsh sickness in the next infection with other serotypes. Immunity after infection is type-specific, and immunity for all serotypes is maintained for a short time [4].

Nevertheless, the viruses are cross-reactive, there is no cross-protective immunity; a person could be infected with DF up to four times, given the opportunity for different serotypes of the illness, ranging from DF to DHF and DSS [5,6]. DF transmits from one person to another by bites from the *Aedes* mosquito, which receives the virus from an infected person. The virus replicates inside the mosquito and is transmitted to another person whom the mosquito bites. This type of mosquito is prevalent in urban areas because the *Aedes aegypti* mosquito lays its eggs in water containers rather than in ponds or ditches. During the epidemic in USA in 2001, it was discovered that the *Ae. albopictus*, *Ae. polynesiensis*, *and Ae. Scutellar* mosquitoes can also transmit DF, while only *Ae. albopictus* was found during the Hawaiian outbreak [5,7,8].

2. History of dengue fever

The first report for a sickness similar to DF was in the late eighteenth century, and it was a temporary outbreak. It is assumed that DF was initially transmitted by travel and trade shipping between Asia and South America. The DF pandemic that occurred following World War II was due to environmental damage, displaced people, and discarded military equipment, which became hosts for vector reproduction. In the twentieth century, DHF/DSS was identified as a diagnostic sign for dengue infection. Afterwards, cases have increased internationally, and dengue disease has been transmitted to other areas of the world [8].

Deadly dengue infection was first documented in an outbreak in Manila, Philippines, in 1953–54. For 10–15 years, it developed to being endemic throughout Southeast Asia. The first epidemic in Singapore was in 1960 and infected older children and adults, with a low mortality rate. In 1996, a harsh outbreak of dengue/DHF occurred in Delhi, and approximately 10,252 cases were reported; there were 423 deaths [7].

Associated work started in 1949 to minimize mosquito reproduction, which subsequently decreased the number of DF cases. Nevertheless, reduction in the use of the anti-insect dichlorodiphenyltrichloroethane (more commonly known as DDT) caused a rise in DF cases in 1970, and it was one of the most pandemic diseases [5].

Although the WHO data between 1992 and 1998 show that Southeast Asia has had a stable number of DF cases, the rate of cases in the Western Pacific has increased four-fold. The numbers in the Americas increased eight-fold during the same period. WHO in 1980 stated that 1,033,417 is the number of DF in the American region. By 2002, that number had climbed to 8,491,416. At present, approximately 100 countries have endemic DF, with 100 million cases predicted yearly [5].

3. Geographic distribution of the disease

The regions with the maximum disease infections are in the Asia–Pacific region, both central and south America and southeast of the Gulf of Mexico. Dengue is found in Africa but is less frequent there. In 2009, dengue re-emerged in Florida after a 75-year absence. In 2010, epidemics have been declared in the Philippines, the Caribbean, Central America and Sri Lanka. It is expected that, in 2020, the number of travelers could rise with international arrivals reaching some 1.6 billion, especially in common tourist destinations [4].

DF reproduces in subtropical and tropical regions because climate affects the reproduction and the incubation stage of the DENV in the *Aedes* mosquito, and the harshness of the epidemic. A congested urban region with inappropriate hygiene can affect outbreaks of DF [5]. Global warming and climate change could help the spread, the prevalence, and the geographic range of DF. Warming global temperatures boost the risk of contact with infections by increasing the rainfall and the habitat for vectors [5].

4. Dengue-fever symptoms

DF presents with different symptoms, from basically no symptoms to severe disease. A second infection with another serotype of the virus can cause an endothelial leak and bleeding, which are symptoms of DHF. A small number of patients with DHF can develop circulatory failure and refractory shock, known as DSS, which could be fatal. However, DHF and DSS based on the disease serotype perhaps occur with a first dengue attack [7]. The mortality rate for (DSS) dengue is quoted as being ~1–5% [4]. The incubation period of DF after the mosquito bite is usually 4–7 days, and the range 3–14 days. Typically, the disease appears with a high fever that could reach 39°C or higher [7].

Usually, there are also spots, nausea and vomiting, and harsh muscular pains and headache could be present [4,6]. DHF is known mainly through the hemoglobin concentration, hemorrhagic symptoms and vascular leakage, and could subsequently end the patient's life. The disease development to DHF is inadequately known; however, it could develop because of the viral virulence antigen, host hereditary and acquired factors, multiple infections and immune pathological reactions [9].

5. Methods for diagnosing dengue fever

There are several types of tests for detecting the DENV. The traditional diagnostic techniques for the DENV is its isolation in cell culture, serological testing, PCR and, more recently, biosensors and fast methods.

5.1. Isolation

The conventional test to identify DENV is virus isolation in a cell culture or live mosquitoes, which was the preferred test in the last century [10,11]. Isolated viruses can be used for virological analyses, which could also give molecular epidemiological information by analyzing isolated viruses. Thus, virus isolation can provide