Study of Dengue virus by Serological test and Rapid PCR

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Abstract

Dengue fever is a viral infection spread by arthropods and caused by four antigenically distinct dengue virus serotypes. Around the world, this disease is regarded as a major public health concern. Dengue fever is currently without an approved vaccine or antiviral drug for prevention and treatment. Furthermore, the clinical signs and symptoms of dengue are similar to those of malaria, chikungunya, rickettsia, and leptospira. As a result, for disease confirmation and patient selection, a quick and accurate laboratory diagnostic test is critical. Traditional dengue virus diagnostic procedures include viral detection in cell culture, serological testing, and reverse transcriptase PCR amplification. The standard laboratory techniques for identifying dengue fever during the acute and convalescent phases are described in this publication. Then, utilizing specific transducers, biosensor-based assays for the detection of dengue disease are investigated.

Keywords: Dengue, diagnosis, detection, biosensor, PCR, dengue specific IgM/IgG, NS1, viral isolation

Introduction

DENV is a flavivirus disseminated largely by the female Aedes Aegyptus mosquito, which is found primarily in urban centers [1]. The first virologically confirmed case of dengue fever in the United States occurred in Philadelphia in 1780 [2]. However, the first dengue-like symptoms were described in the Americas in 1635 [2]. The incubation period within a non-infected mosquito lasts 4–10 days after it bites an infected human. After the incubation stage, the vector might continue to transmit the virus for the rest of its life. [3]. DENV is divided into four serotypes that are genetically and antigenically linked, as follows: DENV-1, 2, 3, and 4 [4,5]. All four DENV serotypes are mutating in subtropical regions of Asia, Africa, Europe, North and South America. DENV normally causes a flu-like sickness in people of all ages, although more serious disease symptoms, such as plasma leakage, are also prevalent [6,7]. A high fever of roughly 104 degrees Fahrenheit, aches behind the eyes, severe headache, muscle/joint discomfort, vomiting, inflamed glands, and rash are all common infection symptoms [8]. These symptoms can persist for up to seven days, but they usually develop four to ten days after the person is bitten by a mosquito. These symptoms can persist for up to seven days, but they usually develop four to ten days after the person is bitten by a mosquito [9]. Plasma spilling, fluid accumulation, ascites, pleural effusions, severe bleeding, low platelets, and/or organ damage can all make severe dengue fever (DF) potentially fatal [10]. Despite this, patients infected with DENV-2 and DENV-4 get acute dengue hemorrhagic fever (DHF), but infection with DENV-1 and DENV-3 is mild and sometimes undetectable. According to WHO criteria, DHF can be classified into four grades: grade I am moderate bruising, grade II is spontaneous blood loss into the skin and elsewhere, grade III is shock symptoms, and grade IV is acute shock [11]. There is currently no gold standard antiviral treatment for DF/DHF, however, maintaining a patient's bodily fluids is critical in the treatment [12]. Only one vaccine has been approved for use in numerous countries so far, but it can only be given to persons who have already been infected [13]. DENV diagnostic testing differs in terms of form, cost, and duration. The virus is detected using both nucleic acid amplification assays (NAATs) and serologic tests [14].Monitoring viral load by detecting genomic nucleic acids in infected patients is the gold standard for diagnosing dengue infection in its early stages [15]. The DENV NS1 protein, on the other hand, has clinical relevance since it helps individuals to detect the virus in its early stages (0 to 14 days). The immune response to the viral infection begins to appear within a few days of symptom onset [16]. As a result, the IgM and IgG responses of the immune system are being studied as a potential dengue virus diagnostic medium. Furthermore, because DENV infection co-circulates with other flaviviruses, including the Zika virus, DENV specific identification is critical in DENV outbreak management [17]. Traditional techniques of diagnosis necessitate sophisticated medical facilities as well as highly trained individuals. Although DENV is predominantly distributed in nations with limited resources, the majority of the current tests are not adequate for resource-constrained settings [18]. As a result,using fluorescence, colorimetric, or impedimetric detection principles, many state-of-the-art biosensing technologies have recently been developed for the low-cost and quick detection of DENV. The application of these biosensor-based tests necessitates a smaller sample size. These biosensors are a good alternative to traditional lab-based assays for early detection because of their high sensitivity and specificity, as well as their portability. Multiplexed assays have also been reported to be useful in detecting and distinguishing DENV from other flaviviruses in areas where DENV is endemic.

The current common technologies used to test DENV in resource-limited settings are discussed in this study. We also examine, analyze, and compare their results to those of currently being developed or newly commercialized methods [20]. Furthermore, we review current state-of-the-art biosensing technologies, evaluate their performance, present solutions to solve disease-related problems and propose future guidance for improved diagnostic utilization during DENV recurrence or future outbreaks.This study is thorough, including both laboratory-based conventional and point-of-care diagnostic modalities and filling in information gaps not addressed in other recent reviews. We concentrate on current advances in POC-based biosensing assays that have been developed since 2015 [21]. We also discuss current advances in dengue sensing, such as CRISPR-based approaches, which have not previously been discussed [22-24].

LABORATORY DIAGNOSTIC TESTS

Serological Tests

In comparison to molecular or culture-based testing, serological assays are the most extensively employed to identify dengue infection because of their low cost and ease of use. The most common specimens used to test for IgM and neutralizing antibodies are serum and cerebrospinal fluid. Plasma and whole blood samples, on the other hand, are occasionally utilized as testing specimens. The presence of the IgG antibody can potentially be used as a dengue biomarker. After a first infection, IgG levels remain in the human body. It can produce equivocal results for secondary infection and false-positive results in people who have been infected with or have been immunized against other flaviviruses.

IgM-Based Tests

Antibodies are proteins produced by the immune system in response to an antigen attack. Immunoglobulin proteins, such as Ig (G, M, A, E, and D), are typical antibodies that are generated in response to antigens by B-cells and T-cells [25]. IgM antibodies are created five days after the onset of symptoms during infection and remain in the human patient's body for two to three months, sometimes even longer. The main issue with Dengue antibody detection devices is that samples are taken before the five-day negative window period.The IgM antibody response is higher in first-time dengue infections, while the IgG antibody response is higher in second-time dengue infections [26-29]. To detect IgM antibodies in dengue, the Armed Forces Research Institute of Medical Sciences developed an enzyme-linked immunosorbent assay capturing the IgM antibody test. Anti-human IgM antibodies on a solid phase catch the dengue IgM antibody from the patient's serum specimen to perform the test.The incubation period for dengue fever is normally five days, and the acute illness appears between days 0 and 7, with some individuals experiencing hemorrhagic shock after that time. RT-PCR or any other molecular detection technology can be used to evaluate viral load during the early stages of infection [30-34]. NS1 can also be detected using the ELISA approach up to 10 days after infection. After a few days of infection, IgM or IgG-based tests can be used to detect dengue infection.

IgG-Based Tests

Past and present infections can be determined using IgG-based assays. IgG antibodies are formed after IgM antibodies, and they might last for a longer time, even a lifetime [35].The IgG-based ELISA from Standard Diagnostics has a sensitivity of 81.2 percent and a specificity of 39.8%. These tests were compared to their NS1 and IgM/IgG antibodies in a study, and it was discovered that the IgG tests had a high rate of false positives and strong cross-reactivity [36,37]. In some cases, IgG tests are useful, although IgM and NS1 ELISA are more useful for acute infections. Because of cross-reactivity with other flavivirus IgG antibodies, determining primary infection with IgG-based assays is difficult [38].

IgM/IgG Ratio Tests

An IgM/IgG ratio test is commonly used to distinguish between first-time and subsequent infections. In both IgM and IgG ELISA tests, the optical density (OD) is assessed [39]. Primary infection is defined as a ratio more than 1.32, whereas secondary infection is defined as a ratio less than 1.32. The cut-off value of the OD ratio is frequently regulated by specific laboratories to determine the infection's outcome. The optimal cut-off ratio, according to one laboratory in Northern India, is 1.1. Both IgM and IgG can be used to figure out what kind of infection is present. The cut-off ratio will differ depending on the IgM and IgG tests done [40].

NS1 Based Tests

The antigen Non-structural 1 (NS1) is important for DENV replication in the host cell. Because the antigen is produced and released into the bloodstream of infected patients, it is regarded as a critical biomarker for diagnosing flavivirus infection early on. NS1 assays are particularly useful in clinical settings because they can detect the DENV's acute phase, and NS1 lasts longer in the blood than viremia. In most cases, rapid detection systems are lateral flow-based rapid tests, and laboratory-based testing is done with antigen-capture ELISAs. The NS1-based assays, according to the CDC, provide similar results to molecular tests in the first week of infection [41-46]. A positive NS1 result confirms DENV infection, whereas a negative result does not rule out infection. If the test results are negative, an IgM-based test should be done. The fact that NS1-based testing is not indicated beyond seven days is a

significant disadvantage.

Method for NS1 and IgG/IgM

The SD BIOLINE Dengue Duo Rapid Test includes two in vitro immune-chromatographic tests for detecting dengue infection in human serum, plasma, or whole blood. The Dengue NS1 antigen Rapid Test is a one-step assay for the qualitative detection of dengue virus NS1 antigen that is highly sensitive and specific. When NS1 antigen is injected into the specimen well, it reacts with mouse monoclonal anti-dengue Ab-colloidal gold conjugates, resulting in a combination of antibodies and antigen. By chromatography, this combination is collected by immobilized antibodies as it migrates along with the test device, resulting in a colored Test Line near to "T" [47].If the test technique is conducted successfully and the active ingredients of the primary components on the strip are working, a second line is utilized for procedural control and should always appear adjacent to "C" (Control Line). Both the Control ("C") and Test ("T") Lines display in the result window when the dengue virus NS1 antigen is present in the samples, indicating a positive result. For specimens that do not contain NS1 antigen or have NS1 antigen at levels below detectable levels, only the Control Line ("C") shows, indicating a negative result [48]. The Dengue IgG/IgM Rapid Test is a qualitative assay that distinguishes between IgG and IgM antibodies to the dengue virus. Using a combination of recombinant dengue envelope proteins, this test identifies antibodies for all four dengue serotypes. Anti-dengue IgG and IgM present in the specimen react with the recombinant dengue virus envelope proteins colloidal gold conjugates when introduced to the specimen well, generating a complex of antibodies and antigens.This complex is caught by immobilized anti-human IgG and/or anti-human IgM as it migrates along with the test device, resulting in two-colored Test Lines adjacent to "G" (Dengue IgG Test Line) and/or "M" (Dengue IgM Test Line) (Dengue IgM Test Line). If the test method is conducted successfully and the active ingredients of the primary components on the strip are working, a third line is utilized for procedural control and should always appear adjacent to "C" (Control Line). Both the Control ("C") and Test ("G" and/or "M") Lines display in the result window when dengue antibodies are present in the samples, indicating a positive result [49]. For specimens that do not contain dengue IgG/IgM ("G"/"M") or that contain these antibodies below the detection limit, only the Control Line ("C") appears.

Fig 1: Dengue duo kit

Methods	Positive case detected	Percentage
Ns-1	10	25%
IgM/IgG	14	35%
PCR	16	40%

Table 1: Shows the no. of positive cases by different diagnostic methods.

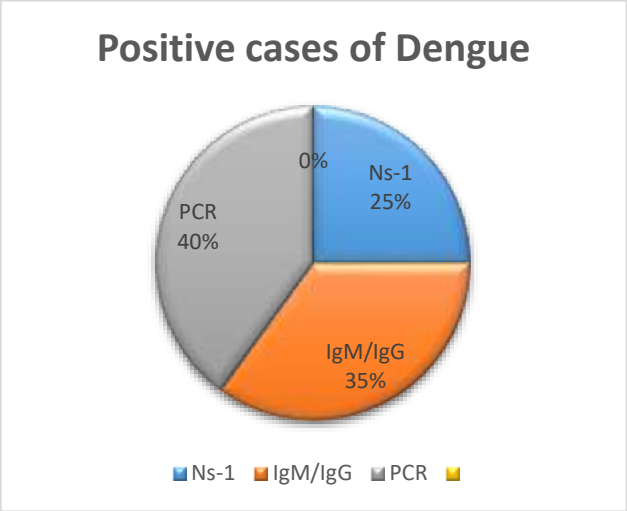

Fig.2: Shows the no. of positive cases by different diagnostic methods.

Molecular Detection

Nucleic acid tests (NATs) are molecular assays performed in a centralized lab setting that necessitate high-tech equipment and highly trained personnel. NATs are more accurate and sensitive at diagnosing and quantifying viral RNA/DNA, allowing identification in the acute phase. Within five days of the onset of symptoms, the viral genome can be discovered. Several assays have already been devised that can detect quantitatively, semi-quantitatively, or qualitatively.

Polymerase Chain Reaction (PCR) Based Tests

PCR is the most frequent DENV NAT test, and because of its superior sensitivity, it is also regarded as the gold standard for detecting DENV at an earlier stage of illness [53]. The viral RNA is isolated from several sources, including plasma, blood, urine, and serum, in RT-PCR. The viral RNA is then converted to cDNA, amplified, and the fluorescence of the amplified RNA is read by devices to detect positive and negative outcomes.The Truenat KFD system (Molbio Diagnostics Pvt.Ltd.) is made up of three parts: (1) an AutoPrep sample prep kit; (2) a Trueprep AUTO RNA extraction system; and (3) a Truelab UnoDx PCR analyzer system. KFDV clinical specimens were handled with normal personal protective equipment and procedures, as well as BSL-3/BSL-2 biosafety measures [54]. For inactivation, a 50-liter extraction volume from suspicious specimens (mostly human blood, serum, homogenized monkey necropsy organs, and homogenized tick pools) was treated with lysis buffer. The treated material was placed into an RNA extraction cartridge and subsequently into the Trueprep AUTO RNA extraction equipment. Each sample received 150 l of RNA elute, of which 6 l was added to a reagent tube containing lyophilized master mix [55]. For 30 seconds, the reaction mixture was incubated. 6 l of the reaction mixture was added to the microchip after it was placed on the PCR analyzer tray. The cycle threshold (Ct) value and optical graph were viewed at the end of the 40-minute run.

Conclusion

To summarise, PCR-based molecular diagnosis of Dengue virus shows a lot of possibility for improving clinicians' abilities to diagnose dengue. There have been several initiatives to see if IgM/IgG can replace it as a valuable tool in the laboratory diagnosis of dengue infections. In terms of rapid-routine serological tests, they differ. Commercial kits are beneficial for quick screening, especially in endemic areas where secondary infection is the most common cause of illness. RT-PCR has various advantages over traditional approaches. In serum samples, the RT-PCR was able to detect laboratory-adapted DENV of all serotypes. The current paper describes the creation of a simple, specific, and highly sensitive RT-PCR that could be used for routine laboratory diagnosis of a variety of diseases.

Acknowledgment

It's our privilege and honor to express our sincerest gratitude to the Parul University, Vadodara, Gujarat for providing me with all the necessary support and facilities including state-of-the-art infrastructure facilities with advanced technological scientific laboratories and everything else that was required to carry out this.

Conflicts of interest

The authors declare no conflicts of interest.

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