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Clinical assessment of NS1 antigen as an early serological marker in suspected cases of Dengue

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Abstract
These serological tests detect either IgM and/or IgG antibodies or both. Antibody detection tests have low sensitivity for the early diagnosis because even the IgM antibodies start appearing after the 5th day of fever which delays the diagnosis and increases the mortality. Diagnosis of infection during the first week of fever is extremely important in the clinical management of the patient and to prevent potential outbreaks. In order to do so, antigen detection tests came to existence which detect NS1 antigen. This antigen is a non-structural protein which is a highly conserved protein which can be detectable during the very early phase of the disease. The levels of NS1 antigen in the circulation is very high in the acute phase of the disease and can be detected as early as day 1 of the fever. NS1 antigen is also highly specific in the diagnosis of dengue infection. Hence based on above background the present study was planned to know the clinical assessment of NS1 antigen as an early serological marker in suspected cases of dengue.
The present study was planned in Department of Microbiology, ANMMC Gaya, Bihar from July 2018 to march 2019. Total 50 cases identified with symptoms of the dengue were enrolled in the present study. The cases were analysed for dengue serological markers. All the serum samples were screened for NS1 antigen and IgG and IgM antibodies by using lateral flow immunochromatographic rapid test. Platelet counts were detected by automated analyzer and duration of fever has been noted down. In India the problem of dengue is complex due to the large population, poor medical and diagnostic facilities and inadequate mosquito control. This country needs a quick effective technique and reliable diagnosis in addressing this potentially fatal, epidemic prone infection and large number of virus laboratories to confirm it and alert the public to take action. In conclusion, this study suggests that NS1 assays deserve inclusion in the diagnostic evaluation of dengue patients, but with due consideration for limitations in patients who present late in their illness or have a concomitant humoral immune response.

Keywords: NS1 antigen, dengue, serological markers, etc

Introduction
Dengue fever is a mosquito-borne tropical disease caused by the dengue virus. Symptoms typically begin three to fourteen days after infection. This may include a high fever, headache, vomiting, muscle and joint pains, and a characteristic skin rash. Recovery generally takes two to seven days. In a small proportion of cases, the disease develops into severe dengue, also known as dengue hemorrhagic fever, resulting in bleeding, low levels of blood platelets and blood plasma leakage, or into dengue shock syndrome, where dangerously low blood pressure occurs [1 2].
Dengue is spread by several species of female mosquitoes of the Aedes type, principally A. aegypti. The virus has five types; infection with one type usually gives lifelong immunity to that type, but only short-term immunity to the others. Subsequent infection with a different type increases the risk of severe complications. A number of tests are available to confirm the diagnosis including detecting antibodies to the virus or its RNA [2].
A vaccine for dengue fever has been approved and is commercially available in a number of countries. The vaccine, however, is only recommended in those who have been previously infected. Other methods of prevention include reducing mosquito habitat and limiting exposure to bites. This may be done by getting rid of or covering standing water and wearing clothing that covers much of the body. Treatment of acute dengue is supportive and includes giving fluid either by mouth or intravenously for mild or moderate disease. For more severe cases, blood transfusion may be required. About half a million people require hospital admission every year. Paracetamol (acetaminophen) is recommended instead of nonsteroidal anti-inflammatory drugs (NSAIDs) for fever reduction and pain relief in dengue due to an increased risk of bleeding from NSAID use [3].
Typically, people infected with dengue virus are asymptomatic (80%) or have only mild symptoms such as an uncomplicated fever. Others have more severe illness (5%), and in a small proportion it is life-threatening. The incubation period (time between exposure and onset of symptoms) ranges from 3 to 14 days, but most often it is 4 to 7 days. Therefore, travellers returning from endemic areas are unlikely to have dengue if fever or other symptoms start more than 14 days after arriving home. Children often experience symptoms similar to those of the common cold and gastroenteritis (vomiting and diarrhea) and have a greater risk of severe complications, though initial symptoms are generally mild but include high fever [4]
. The characteristic symptoms of dengue are sudden-onset

fever, headache (typically located behind the eyes), muscle and joint pains, and a rash. The alternative name for dengue, "breakbone fever", comes from the associated muscle and joint pains. The course of infection is divided into three phases: febrile, critical, and recovery.

The febrile phase involves high fever, potentially over 40°C (104°F), and is associated with generalized pain and a headache; this usually lasts two to seven days. Nausea and vomiting may also occur. A rash occurs in 50–80% of those with symptoms in the first or second day of symptoms as flushed skin, or later in the course of illness (days 4–7), as a measles-like rash. A rash described as "islands of white in a sea of red" has also been observed. Some petechiae (small red spots that do not disappear when the skin is pressed, which are caused by broken capillaries) can appear at this point, as may some mild bleeding from the mucous membranes of the mouth and nose. The fever itself is classically biphasic or saddleback in nature, breaking and then returning for one or two days [5-6].

In some people, the disease proceeds to a critical phase as fever resolves. During this period, there is leakage of plasma from the blood vessels, typically lasting one to two days. This may result in fluid accumulation in the chest and abdominal cavity as well as depletion of fluid from the circulation and decreased blood supply to vital organs. There may also be organ dysfunction and severe bleeding, typically from the gastrointestinal tract. Shock (dengue shock syndrome) and hemorrhage (dengue hemorrhagic fever) occur in less than 5% of all cases of dengue; however, those who have previously been infected with other serotypes of dengue virus ("secondary infection") are at an increased risk. This critical phase, while rare, occurs relatively more commonly in children and young adults [7].

The recovery phase occurs next, with resorption of the leaked fluid into the bloodstream. This usually lasts two to three days. The improvement is often striking, and can be accompanied with severe itching and a slow heart rate. Another rash may occur with either a maculopapular or a vasculitic appearance, which is followed by peeling of the skin. During this stage, a fluid overload state may occur; if it affects the brain, it may cause a reduced level of consciousness or seizures. A feeling of fatigue may last for weeks in adults [7].

The diagnosis of dengue fever may be confirmed by microbiological laboratory testing. This can be done by virus isolation in cell cultures, nucleic acid detection by PCR, viral antigen detection (such as for NS1) or specific antibodies (serology). Virus isolation and nucleic acid detection are more accurate than antigen detection, but these tests are not widely available due to their greater cost. Detection of NS1 during the febrile phase of a primary infection may be greater than 90% sensitive however is only 60–80% in subsequent infections. All tests may be negative in the early stages of the disease. PCR and viral antigen detection are more accurate in the first seven days. In 2012 a PCR test was introduced that can run on equipment used to diagnose influenza; this is likely to improve access to PCR-based diagnosis [8].

There are no specific antiviral drugs for dengue; however, maintaining proper fluid balance is important. Treatment depends on the symptoms. Those who are able to drink, are passing urine, have no "warning signs" and are otherwise healthy can be managed at home with daily follow-up and oral rehydration therapy. Those who have other health problems, have "warning signs", or cannot manage regular follow-up should be cared for in hospital. In those with severe dengue care should be provided in an area where there is access to an intensive care unit. Intravenous hydration, if required, is typically only needed for one or two days. In children with shock due to dengue a rapid dose of 20 mL/kg is reasonable [9]. The rate of fluid administration is then titrated to a urinary output of 0.5–1 mL/kg/h, stable vital signs and normalization of hematocrit. The smallest amount of fluid required to achieve this is recommended.

Invasive medical procedures such as nasogastric intubation, intramuscular injections and arterial punctures are avoided, in view of the bleeding risk. Paracetamol (acetaminophen) is used for fever and discomfort while NSAIDs such as ibuprofen and aspirin are avoided as they might aggravate the risk of bleeding. Blood transfusion is initiated early in people presenting with unstable vital signs in the face of a decreasing hematocrit, rather than waiting for the hemoglobin concentration to decrease to some predetermined "transfusion trigger" level. Packed red blood cells or whole blood are recommended, while platelets and fresh frozen plasma are usually not. There is not enough evidence to determine if corticosteroids have a positive or negative effect in dengue fever [10].

During the recovery phase intravenous fluids are discontinued to prevent a state of fluid overload. If fluid overload occurs and vital signs are stable, stopping further fluid may be all that is needed. If a person is outside of the critical phase, a loop diuretic such as furosemide may be used to eliminate excess fluid from the circulation [11].

These laboratory tests are only of diagnostic value during the acute phase of the illness with the exception of serology. Tests for dengue virus-specific antibodies, types IgG and IgM, can be useful in confirming a diagnosis in the later stages of the infection. Both IgG and IgM are produced after 5–7 days. The highest levels (titres) of IgM are detected following a primary infection, but IgM is also produced in reinfection. IgM becomes undetectable 30–90 days after a primary infection, but earlier following re-infections. IgG, by contrast, remains detectable for over 60 years and, in the absence of symptoms, is a useful indicator of past infection. After a primary infection, IgG reaches peak levels in the blood after 14–21 days. In subsequent re-infections, levels peak earlier and the titres are usually higher. Both IgG and IgM provide protective immunity to the infecting serotype of the virus. In testing for IgG and IgM antibodies there may be cross-reactivity with other flaviviruses which may result in a false positive after recent infections or vaccinations with yellow fever virus or Japanese encephalitis. The detection of IgG alone is not considered diagnostic unless blood samples are collected 14 days apart and a greater than fourfold increase in levels of specific IgG is detected. In a person with symptoms, the detection of IgM is considered diagnostic [7-12].

NS1 antigen test (nonstructural protein 1), is a test for dengue, introduced in 2006. It allows rapid detection on the first day of fever, before antibodies appear some 5 or more days later. It has been adopted for use in some 40 nations. The method of detection is through enzyme-linked immunosorbent assay [13]. India has introduced in 2010 the NS1 test costing 1,600 rupees at a private hospital in Mumbai [14].

The medical use of the NS1 antigen test can be defined to

diagnose dengue infections and is effective to 1st day detection. Additionally, NS1 assay is useful for differential diagnostics in regards to flaviviruses [15]. NS1 is present in the serum of infected persons directly at the onset of clinical symptoms in primary dengue infection and produces a strong humoral response. It is detectable before the appearance of IgM antibodies [16]. DENV by NS1 antigen is laboratory confirmation of dengue in people also assessing clinical aspects (as well as, taking into account where the individual may have travelled recently) [17]. Serological tests such as an immunoglobulin M antibody capture–enzyme-linked immunosorbent assay (MAC-ELISA) and viral RNA detection by reverse transcriptase (RT-PCR) can also be used to diagnose Dengue fever [18].

These serological tests detect either IgM and/or IgG antibodies or both. Antibody detection tests have low sensitivity for the early diagnosis because even the IgM antibodies start appearing after the 5th day of fever which delays the diagnosis and increases the mortality. Diagnosis of infection during the first week of fever is extremely important in the clinical management of the patient and to prevent potential outbreaks. In order to do so, antigen detection tests came to existence which detect NS1 antigen. This antigen is a non-structural protein which is a highly conserved protein which can be detectable during the very early phase of the disease. The levels of NS1 antigen in the circulation is very high in the acute phase of the disease and can be detected as early as day 1 of the fever. NS1 antigen is also highly specific in the diagnosis of dengue infection [19-22]. Hence based on above background the present study was planned to know the clinical assessment of NS1 antigen as an early serological marker in suspected cases of dengue.

Methodolog

The present study was planned in Department of Microbiology, ANMMC Gaya, Bihar from July 2018 to march 2019. Total 50 cases identified with symptoms of the dengue were enrolled in the present study. The cases were analysed for dengue serological markers. All the serum samples were screened for NS1 antigen and IgG and IgM antibodies by using lateral flow immunochromatographic rapid test. Platelet counts were detected by automated analyzer and duration of fever has been noted down.

All the patients were informed consents. The aim and the objective of the present study were conveyed to them. Approval of the institutional ethical committee was taken prior to conduct of this study.

Following was the inclusion and exclusion criteria for the present study.

Inclusion criteria: All the patients with Clinical features suggestive of Dengue infection, later on confirmed by serology were included in this study.

Exclusion criteria: Any other identified specific infection was excluded from the study.

Results & Discussion:

According to WHO estimates dengue viral infections are responsible for hundred million cases of dengue infections annually and more than five lakh cases of dengue haemorrhagic fever. The main factors which had made dengue infections a global health problem is unprecedented global population growth, unplanned and uncontrolled urbanisation and deterioration in water, sewer and waste management systems. The emerging dengue viral infections is of great threat to the mankind as there is no specific medication available nor there is any vaccine available against dengue virus [23-25].

In serological diagnosis of dengue NS1 antigen is playing a pivotal role to diagnose the disease in its early days. Many physicians depend only on NS1 antigen test for early diagnosis of dengue. NS1 produces a very strong humoral response. NS1 antigen is detected for diagnosing dengue cases from 1st day onwards of the appearance of disease symptoms. NS1 antigen detection may have higher sensitivity during the first 5 days after the onset of symptoms thereafter NS1 antigen decreases gradually and antibody detection tests have higher sensitivity after day 5 of the disease following onset [26]. According to WHO in the patient's serum NS1 antigen is usually found circulating from the first day after the onset of fever up to the 5th day and it may be extended up to 9 days after the onset of infection in some cases [27]. The circulation of NS1 antigen from the 1st day in the patient serum after the onset of fever up to day 9 has been reported by other workers [28].

Table 1: Age Distribution

Age	Number of Cases
0 – 20 years	12
21 – 40 years	15
41 – 60 years	18
Above 60 years	5
Total	50

Table 2: Dengue Marker and No. of Cases

Dengue marker	Number of Cases
NS1	27
IgM	20
IgG	1
NS1 + IgM	1
IgM + IgG	1
Total	**50**

Table 3: Comparison of platelet counts with dengue parameters

Parameter	Total Cases	No. of Cases of Platelet Count Less than 10^5/ml
NS1	27	13
IgM	20	8
IgG	1	1
NS1 + IgM	1	1
IgM + IgG	1	0
Total	**50**	**23**

NS1 antigen as an early diagnostic marker in dengue was reported from India [29]. NS1 antigen test was able to detect dengue virus infection from days 1 to 8 in 44% of samples [30]. According to another study the sensitivity of the NS1, IgM ELISA was higher when compared with RT PCR and therefore it was recommended to use for early diagnosis [31]. NS1 antigen to diagnose early dengue infection was also reported in another study in India [32]. In those studies, mentioned above the proportion of dengue cases positive with NS1 antigen beyond 5 days of infection was actually not available.

In Primary dengue case there will be a low titre and slow rising of antibodies. IgM antibody will appear first after 3-5 day followed by IgG antibody at the end of first week of illness. IgM levels peak about two weeks after the onset of

symptoms and then decline generally to undetectable levels over 2–3 months. But anti-dengue serum IgG is increasing slowly after first week and thereafter it remains detectable after several months, and probably even for life. In contrast during secondary infection, rapid increase and high titre of antibodies are seen, i.e., high levels of IgG can be detected even during acute phase of secondary infection and IgM response is variable [33-34].

In Dengue fever the virus may interact and activate platelets leading to thrombocytopenia or may affect growth and differentiation of thrombopoiet in induced megakaryopoiesis inducing apoptotic cell changes in a subpopulation of early megakaryocytic progenitors. These events might contribute towards the origin of thrombocytopenia in dengue disease.

Conventional ELISA require at least 4 hours whereas rapid immunochromatographic test requires twenty minutes, which will be helpful in initiating the treatment and minimizing the complications and mortality of dengue [35]. But the lack of conformity in the evaluation of Dengue rapid diagnostic tests (RDTs) remains a problem and a standardised approach must be performed when performing diagnostic assessment. Moreover, studies have demonstrated that Rapid diagnostic test cannot reliably differentiate the different dengue infection states (primary or secondary dengue infection). The manufacturers of the kit used in this study allows the use of serum, plasma or whole blood for use in dengue rapid diagnostic tests. [36] Unfortunately there is little evidence that all sample types perform equally well. The effect of anticoagulants and whole blood on RDT performance and ease of reading also require examination in a field setting [37].

Conclusion:

In India the problem of dengue is complex due to the large population, poor medical and diagnostic facilities and inadequate mosquito control. This country needs a quick effective technique and reliable diagnosis in addressing this potentially fatal, epidemic prone infection and large number of virus laboratories to confirm it and alert the public to take action. In conclusion, this study suggests that NS1 assays deserve inclusion in the diagnostic evaluation of dengue patients, but with due consideration for limitations in patients who present late in their illness or have a concomitant humoral immune response.

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