International Journal of Medical and Health Research
ISSN: 2454-9142
Received: 10-09-2018; Accepted: 13-10-2018
www.medicalsciencejournal.com
Volume 4; Issue 11; November 2018; Page No. 55-57

Clinical assessment of anti-dengue IgM antibodies in suspected dengue cases in ANMMC, Gaya, Bihar

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Abstract
Laboratory diagnosis of dengue virus infection is based mainly on serological tests. They are relatively inexpensive and easy to perform as compared to culture and nucleic acid based methods. In the primary infection, anti-dengue IgM antibodies are produced from fifth day of infection and remain in circulation for 60-90 days. Anti-dengue IgG antibodies are produced after one week, attain maximum after 2-3 weeks and remain lifelong in circulation. In secondary infection, IgG antibodies are detected at high levels in acute stage while IgM antibodies are present at low levels.
The present study was conducted in Department of Microbiology, Anugrah Narayan Magadh Medical College (ANMMC), Gaya, Bihar, India. The 1350 patients suspected with dengue fever from October 2017 to September 2018 the study period of 12 months were included in the study.
A single blood sample approximately 2-3 ml was collected from each patient in a plain vacutainer tube with all aseptic precautions. Serum was subjected to IgG and IgM anti dengue antibodies by dengue IgG capture enzyme linked immunosorbent assay (ELISA) and dengue IgM capture ELISA.
From the present study it can be concluded that the serological prevalence of the dengue fever in the patients admitted to ANMMC Gaya is 7.41%. In the affected populations had majority of males and mid aged populations. The maximum cases are from monsoon season. This may be due to the increases breading of mosquitoes during the rains. Early diagnosis of infection can prevent life threatening complications and immunological assays such as the IgM capture ELISA is a reliable method for diagnosis of dengue virus infection.

Keywords: anti-dengue, IgM antibodies, clinical assessment, ANMMC, Gaya, Bihar

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 [1]. In a small proportion of cases, the disease develops into the life-threatening dengue haemorrhagic 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].
Dengue is spread by several species of mosquito 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 [1]. 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. Other methods of prevention are by 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 admission to hospital a 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 [2, 3].
Dengue has become a global problem since the Second World War and is common in more than 110 countries. Each year between 50 and 528 million people are infected and approximately 10,000 to 20,000 die. The earliest descriptions of an outbreak date from 1779. Its viral cause and spread were understood by the early 20th century. Apart from eliminating the mosquitoes, work is ongoing for medication targeted directly at the virus. It is classified as a neglected tropical disease [4].
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 diarrhoea) and have a greater risk of severe

complications, though initial symptoms are generally mild but include high fever [5].

Diagnos Dengue card test is based on immunochromatographic test principle for the differential detection of Dengue IgM/IgG antibodies.

Dengue IgM/IgG test device contains three lines; "C" (Control line), "M" (IgM test line) & "G" (IgG test line). IgM test line is coated with anti-human IgM and IgG test line is coated with anti-human IgG. When a sample is added to the device, IgG and IgM antibodies in the sample react with red particles coated with Dengue proteins. As this sample/particle mixture migrates along the length of the test, the anti-dengue IgG or IgM antibody particle complex is captured by the relevant IgG and /or IgM test bands located in the test device window causing a pale to dark red band to form at the IgG or IgM Region of the test device window. The intensity of the test bands in the device will vary depending upon the amount of antigen /antibody present in the sample. The appearance of any pink/red colour in a specific test region should be considered as positive for that particular antigen and/or antibody type (IgG or IgM). A red procedural control line should always develop in the test device window to indicate that the test has been performed properly.

Laboratory diagnosis of dengue virus infection is based mainly on serological tests. They are relatively inexpensive and easy to perform as compared to culture and nucleic acid based methods. In the primary infection, anti-dengue IgM antibodies are produced from fifth day of infection and remain in circulation for 60-90 days. Anti-dengue IgG antibodies are produced after one week, attain maxima after 2-3 weeks and remain lifelong in circulation. In secondary infection, IgG antibodies are detected at high levels in acute stage while IgM antibodies are present at low levels [5].

Methodology

The present study was conducted in Department of Microbiology, Anugrah Narayan Magadh Medical College (ANMMC), Gaya, Bihar, India. The 1350 patients suspected with dengue fever from October 2017 to September 2018 the study period 12 months were included in the study. The approval of the institutional ethical committee was taken before conduct of this study. All patients were informed consents.

A single blood sample approximately 2-3 ml was collected from each patient in a plain vacutainer tube with all aseptic precautions. Serum was subjected to IgG and IgM anti dengue antibodies by dengue IgG capture enzyme linked immunosorbent assay (ELISA) and dengue IgM capture ELISA.

Results & Discussion

Out of total 1350 patients suspected with the dengue patients 100 were found positive. The data from the 100 patients suspected with the dengue were collected and presented as below. The table 1 shows the age group of the patients enrolled into the study.

Table 1: Age group

Age group	No. of Cases
Less than 20 years	18
21-30 years	42
31-40 years	18
41-50 years	6
51-60 years	7
More than 60 years	9
Total	100

Table 2: Monthly Distribution

Month	No. of positive cases
October 17	20
November 17	15
December 17	7
January 18	4
February 18	2
March 18	3
April 18	2
May18	0
June18	2
July18	9
August18	14
September18	22
Total	100

Table 3: Clinical Manifestation in Positive cases

Characteristics	No. of positive cases
Mild Febrile syndrome	100
Thrombocytopenia	96
Severe Headache	72
Nausea and Vomiting	65
Myalgia	53
Rash	42
Conjunctiva haemorrhage	29
Leukopenia	22
Pain in abdomen	21
Gingival bleeding	15
Arthralgia	14
GI bleed	13
Retro orbital pain	7
Haemorrhagic manifestations	8

In the present study, 7.41% of the total patients had serologically confirmed dengue infection. Other studies by Turbadkar *et al.* [6] and Ghosh *et al.* [7] from Mumbai reported prevalence of dengue infection as 13.6% and 17.9%, respectively.

Predominant infection rate in adult population was also noted by Kumar A *et al.* [8] and Gupta E *et al.* [9] However, Garg *et al.*, [10] Gunasekaran P *et al.* [11] and Martin JLS *et al.* [12] reported higher prevalence of dengue infection in paediatric age group. A study was conducted at the University of Oxford by Bhatt *et al.*., to estimate the global distribution of dengue cases. Worldwide, 96 million people suffered each year from apparent infections. Asia contributed 70% of this burden due to large areas of densely populated regions which favour

transmission of infection. India had the largest number of dengue cases with about 33 million apparent and another 100 million asymptomatic infections occurring annually. [13]

We included only detection of IgM antibodies against dengue virus in our study and other parameters like IgG antibodies and NS1 antigen detection were not studied. This could be the reason for comparatively lower seroprevalence rate of dengue infection in our study.

Among the clinical signs, we had found that a higher proportion of febrile syndrome, thrombocytopenia, severe headache, nausea, vomiting, myalgia and rash in patients identified with dengue fever.

As per WHO classification, the proposed probable diagnosis – an acute febrile illness with two or more of the following manifestation- headache, retro-orbital pain, arthralgia, rash, haemorrhagic manifestations, leucopoenia and a positive IgM antibody test on serum samples collected five or more days after the onset of fever supports the diagnosis of dengue. [14]

Conclusion

From the present study it can be concluded that the serological prevalence of the dengue fever in the patients admitted to ANMMC Gaya is 7.41%. In the affected populations had majority of males and mid aged populations. The maximum cases are after monsoon season. This may be due to the increases breading of mosquitoes during the rains. Early diagnosis of infection can prevent life threatening complications and immunological assays such as the IgM capture ELISA is a reliable method for diagnosis of dengue virus infection.

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