



IMPACT OF PREVALENT SEROTYPES OF DENGUE VIRUS IN PATIENTS

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ABSTRACT

Dengue has been reported to be endemic in many tropical and sub-tropical countries. Dengue hemorrhagic fever is the major cause of serious illness and death among children in some Asian countries. Our country has also witnessed many outbreaks since the major one in Delhi, 1996. There is currently no specific treatment for Dengue and early signs and symptoms of the disease are similar to those of Malaria, Scrub typhus, Chikungunya and Leptospirosis. Accurate and timely diagnosis is the key to the management of the disease and epidemiological surveillance. The various markers of Dengue infection such as Dengue IgM antibodies, Dengue IgG antibodies, Dengue NS1 antigen and Dengue viral genome have a different and/or overlapping time period of appearance in the blood (WHO Guidelines, 2009). Detection of the viral genome by PCR based technique in acute phase serum is the new gold standard for the detection of infection. Dengue virus has four distinct serotypes. It is also essential to determine which serotypes of Dengue virus are circulating at the given point of time as previous infection with one of the four Dengue serotypes can be an important risk factor for developing DHF-DSS upon infection with a heterotypic serotype. Further molecular detection and serotyping was done in samples of patients with history of fever of less than seven days duration, by conventional reverse transcriptase PCR (RT-PCR).

KEYWORDS: - prevalent serotypes, Dengue virus, patients, Dengue infection, viral genome, Dengue serotypes

INTRODUCTION

Being transmitted by Aedes mosquito, Dengue, a flavivirus has been a great cause of concern in human health in India. Infection caused by dengue virus leads to a complex disease with common clinical symptoms that make it challenging to differentiate it from other common febrile illness during acute phase. Also, this can forge ahead from just mild and nonspecific viral disease to severe cases which could ultimately be characterized by thrombocytopenia, hemorrhage manifestations and other hemo concentration due to plasma leakage (Ali and Ali 2015, Awasthi et al., 2018).

Befalling, more than 100 million of cases and 25000 deaths reported annually, dengue infection has appeared to set 2.5 billion of population at risk (Mustafa, 2011). Dengue virus belongs to the family Flaviviridae and genus Flavivirus. It has positive sense single stranded RNA and is comprise of five serotypes (DENV 1, 2, 3, 4 and 5) with each generating a specific immune response to the infection (Holmes, 1998). All five serotypes of DENV are serologically related, but antigenically distinct. A micrographic image of Dengue virion is shown in Figure 1.

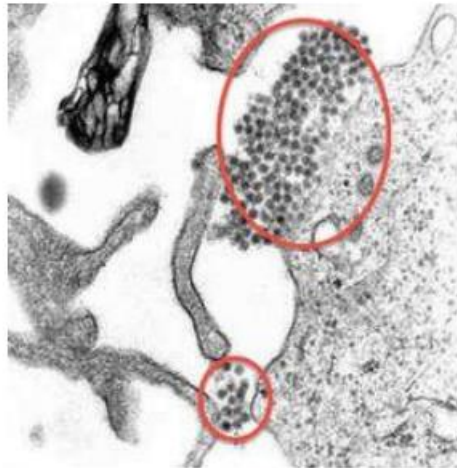


Figure1 Micrographic image showing Dengue virus virions (Yirka, 2015).

Due to dengue virus (DENV), several thousands of people are infected and thereby they contribute to the healthcare burden. These outbreaks of dengue are seasonal and have sustained since 1950s, but the sternness of the disease have grown to extreme extent since past 2 decades. Dengue that is majorly spread between the sub-tropical and tropical regions has become an infection that has led to be a public health concern globally. South-east Asia, Pacific, East and West Africa, America and Caribbean are the countries in which it had been reported endemic. The stagnant water in the bottles, tanks, dumps etc. along with the increased use of non-biodegradable plastics which ultimately become the site for mosquito breeding are generally found near the domestic areas.

DENGUE VIRUS SEROTYPES

Dengue infections are responsible to be caused by DENV-1 to -5. These are five serotypes which arise due to the difference between the antibodies present in the serum of the human. 65% similarity is shared by the five serotypes of dengue virus in their genomes. Although, presence of some genetic variation cause the

between the difference Dengue virus serotypes. These variable serotypes are responsible for the infections which are the results of the same disease with relatable clinical symptoms. The first DENV-1 (DV-1) was firstly secluded at Vellore in the year 1956. The Indian isolates are found to be similar with the Africa and America genotypes. DENV-2 (DV-2) serotypes predominate in India in the 1971 year. This strain was first secluded in India after a 50 years of span time which is from the 1956-2011. DENV-3 (DV-3) strain is emerged in Delhi in 2003. DENV-3 beside with DENV-1 and DENV-2 are allied with DSS/DHF. DENV-4 (DV-4) is the most predominant type of strain in India. It was in 1996, largest dengue outbreak takes place in India due to DENV-2. Then again in 2013, DENV-3 as dominant serotype was responsible for another dengue outbreak. In the October 2013, another serotype named DENV-5 was isolated in India during the viral sample screening. The symptoms shown by this serotype were found to be mild (Mustafa et al.,2015). The viral titer for the secondary infection from the same serotype was found to be four time higher than the other serotypes (Calisher et al., 1989).

MECHANISM

Mosquitoes carry the virus within them and they enter the human body on biting. The entry of virus is caused by the biting mosquito. The viruses bind on WBC where the virus multiplies on reproduction within the cell during their movement all the way through the body. The signaling proteins like cytokines and interferons are produced for making the WBC respond. Due to these signaling proteins, symptoms like fever, severe pains, and the flu-like symptoms arises. When the infection is



severed, large numbers of viruses are produced inside the body which affects many organs like liver and bone marrow. Due to the capillary permeability, fluid is leaked from the blood stream by the means of small blood vessels into body cavities. This cause in low flowing of the blood from the blood vessel and the blood pressure turn out to be very low down as a result and hence sufficient blood cannot be supplied to the organs. Reduced numbers of platelets are observed during the stromal cells infection causing the dysfunction of bone marrow. It causes the bleeding risk and many other major complications related to the dengue fever (Martina, 2009).

REPLICATION

The replication proceeds from the two-step pathway in the host cell. The attachment of dengue virus to the host cell surface takes place which is called the endocytosis process. On reaching in the interior of cell, the fusion of endosomal membrane occurs along with it gets unconstrained within the cytoplasm. The viral particles come spaced out in addition to the release of viral genome. The translation of viral RNA occurs to synthesize single polypeptide which is later on divided into the ten proteins. This is how viral genome is replicated. The endoplasmic reticulum (ER) is responsible for the viral assembly on its surface whilst the budding out of structural proteins with the newly synthesized RNA from the ER happens. The translocation of viral particles transpires in the course of the TGN or the trans-Golgi. These viral particles become matured and then converted in to the infectious form. These mature viruses are now capable for infecting the cell on

releasing and then go on to infect other cells.

Exhibiting a broad range spectrum of clinical profiling, dengue infection sometimes also imparts unpredictable clinical conclusions. Person already infected with the dengue infection, when bitten by a mosquito, it then carries the infected blood and also subsequently when the mosquito bites the other healthy normal individual, and it therefore transmits the infection and spread the disease (WHO 1997; Azin et al., 2012; Puspa et al., 2013). Major transmissions that result into dengue infection are caused by the bites of female mosquitoes (*Aedes* mosquitoes). Most common infection causing vectors for dengue virus are *Aedes aegypti* and *Aedes albopictus*, which lives between the 35°N and 35°S latitudes beneath the altitude 100 meters. Not only humans who serve as the primary host for the virus but also the non-primates also get the infection and the virus circulates through them as well. The most generic infection may be caused by only single bite which is ordinarily during the morning or evening.

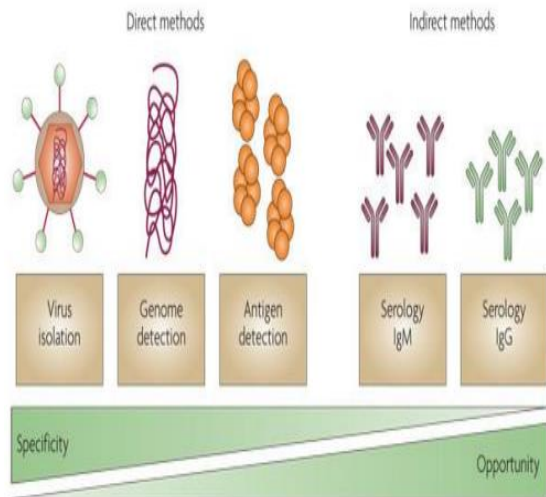


Figure 2 Methods for dengue virus diagnosis and detection [Peeling et al., 2010].

DIAGNOSTIC METHODS

The relevance of the effective and the most accurate diagnosis of dengue is essential for the proper analysis of clinical sample, its surveillance activity, outbreak control, pathogenesis, academic research, and other trails. Several clinical tests can be performed to identify and detect the viral load, the antigen-antibody reaction, and viral nucleic acid. Depicts the information about the various direct and indirect strategies for the detection of dengue infection. The serum sample, plasma sample, or blood cells can be initially taken for the diagnosis of illness in the first 4-5 days of infection. The study of course of dengue illness in detail. Serology method is only chosen for diagnosis after the acute phase of the infection. Whilst for the serotyping and diagnosis, the Real Time-PCR, Reverse transcription PCR, nested PCR, is utilized. These techniques have proven to be quick and sensitive for distinguishing between the several serotypes of the DENV and have replaced the viral isolation techniques.

1 Serological profiling:

It includes the study of serum and other kind of body fluids. It allows the diagnosis and identification of antibodies in the serum.

(i) Antibody Test: It primarily helps in diagnosing the recent or current infection. It detects the presence of two different antibody classes produced against the dengue infection inside the human body which are IgG and IgM. As the body's immune system produces the different amount of antibodies during the disease, diagnosis may include combination of tests.

(ii) NS1 Antigen test (Non-structural protein 1): This test was introduced in the year 2006. The rapid detection is allowed by it on the first onset day of the illness. The infected person contains NS1 in the serum of the person after the onset of clinical symptoms. It can be detected in primary dengue infection. It is responsible for producing a strong humoral response. It can be detected before the production of antibodies, even before the IgM antibodies appear.

(iii) MAC-ELISA: IgM antibodies are used in this method of detection in which the antibody (IgM) is captured on ELISA format. It is widely used in laboratories and a commercially available diagnostic kit is available for the same. The human IgM antibodies are captured using the microtiter plate with the help of anti-human IgM antibody. The Dengue virus specific antigen like DENV-1 to -5 is then added to it. These antigens results from the virus envelope.

(iv) IgG ELISA: This method uses IgG antibody for the detection of secondary dengue infection. The same viral antigens as used in the MAC-ELISA are used in this assay. This method has correlation



with the hem agglutination assay (HI). In the primary dengue infection, the negative IgG is detected in the acute phase whereas a positive IgG is detected in the convalescent phase of the infection. Also, in secondary dengue infection, a positive IgG is detected in the acute infection phase whereas a fourfold rise in IgG titer is observed within the convalescent phase of the infection.

2 Molecular profiling:

It includes the identification and structure documentation of specific DNA, RNA or protein molecule. It is used in diagnosing the disorders related to biochemistry and genetics.

(i) **Molecular Test:** The Dengue virus genetic material is detected from the blood sample in first week of the onset of symptoms appearance like fever. It helps in determining the type of dengue serotype infection. The real-time PCR (RT-PCR) can diagnose the presence of dengue infection and also can distinguish between the dengue infection and Zika and Chikungunya infection. The Centers for Disease Control and Prevention (CDC) provides with the results in four to two weeks of time period. The blood sample cannot be tested after the onset of seven days of illness. An antibody test is suggested if the PCR test came out to be negative.

(ii) **Real Time PCR (RT-PCR):** This method is used to quantify the viral RNA with the use of primers and probes which are very specific to each of the Dengue serotypes. The detection of reaction products in real time is possible by the use of fluorescent probe in the PCR machine called Thermocycler. It does not require electrophoresis for visualizing the results.

There are many assays, which employ TaqMan or SYBR Green Technologies.

TREATMENT

Being a viral disease, dengue has still no specific antibiotic to treat it. Not only antibiotics but also the antiviral medications are not prescribed for dengue infection. Only under physician's supervision, pain relievers such as aspirin and non-steroidal anti-inflammatory drugs should be taken in the severe cases of worsening bleeding complications. Home remedies inclusive of proper rest and fluid intake are the most prescribed and essential. Codeine and Acetaminophen are given in severe headaches and in muscle and joint pains (Sharma et al., 2011; Dash, 2011).

VACCINE

Like any other vaccine that avoids the chances of any sort of infection, dengue vaccine averts the probabilities of the dengue infection. Its development started way before in 1929 but was formerly hindered due to the incomplete pathogenesis knowledge of disease and after that, the need to concurrently build a balanced immunity in response to all the serotypes of dengue. The living attenuated and inactivated vaccines, subunit vaccines, DNA vaccines are being developed. Among all of them, live attenuated vaccines are at the most developmental stage.

PREVENTION

For preventing the spread of communicable disease i.e., dengue infection; several precautionary measures are suggested which majorly impacts in prevention from the mosquito bites during the peak season of infection. Other measures include:



1. Mosquito repellants should be applied to all the exposed areas regularly during day and night in order to protect from dengue.
2. Clean empty flower pots and avoid watering potted plants too much. No stagnant water should be allowed which can become a breeding place for the mosquitoes.
3. Use mosquito net while sleeping (as small children sleep during the day and hence it becomes the prone period for mosquito bite).
4. Plant *Ocimum tenuiflorum* near window to naturally keep out mosquitoes. It also does not allow breeding of the mosquitoes.
5. Check for holes in the windows and door screens. Block the holes to eradicate the mosquito's threat if any.
6. Do not allow collection of excess water by turning over empty pails and buckets or at least cover it when not using.
7. Avoid mosquito bite to the other family members if someone in the family is sick with dengue.
8. While using cooler, remember to clean and throw the water tray regularly even if it is not in use.
9. When trash can or dustbins are not in use, remember to cover them.
10. Camphor also acts as mosquito repellent and hence it should be lighted in the rooms, near doors and windows for fifteen to twenty minutes.

CONCLUSION

Dengue is one of the most rapidly spreading mosquito-borne viral diseases in tropical and subtropical areas. More than 100 countries in the WHO regions of Africa, the Americas, the Eastern Mediterranean, South-East Asia and the Western Pacific are facing the endemicity of the disease. In India, Dengue strikes in

almost every post-monsoon season. The country has witnessed many Dengue epidemics in various states namely Andhra Pradesh, Delhi, Goa, Haryana, Gujarat, Karnataka, Kerala, Maharashtra, Rajasthan, Uttar Pradesh, Pondicherry, Punjab, Tamil Nadu, West Bengal, and Chandigarh. *Aedes aegypti* is the most common vector and transmits Chikungunya, Yellow Fever and Zika infection. Dengue virus can cause a spectrum of illness ranging from self-limiting febrile illness (Dengue fever, DF) to severe and fatal hemorrhagic disease (Dengue with warning signs) and may further lead to circulatory failure (Severe Dengue). There is no specific medicine or antibiotic to treat it, the only treatment is to treat the symptoms. Diagnosis of Dengue can be done by direct methods such as NS1 (Non-Structural protein 1) Antigen detection, viral nucleic acid detection, isolation of virus and indirect methods such as IgM and IgG antibody detection. Patients with dengue like illness; having pyrexia of unknown origin along with one of any signs and symptoms such as nausea or vomiting, rash, arthralgia or retro-orbital pain were included in the study. Samples were received in the laboratory from different outbreak sites all over the state or were self-collected from patients visiting medicine outdoor unit/ admitted to medical units, SMS and attached group of hospitals.

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