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Prevalence and Laboratory Profile of Dengue Viral Infection

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Abstract

Dengue infection is an emerging disease and is a major health problem in our country. Globally the incidence of dengue has increased in the recent years. The WHO estimates that presently about two fifths of the world population is at risk for this viral infection. In 2013 Kerala registered the highest number of cases in India. Standard haematological parameters such as platelet count and haematocrit are important and are part of the biological diagnosis of dengue infection. Therefore, they should be closely monitored. Liver is one of the major target organs involved in acute dengue virus infections; hepatomegaly and increased levels of AST and ALT are common manifestations of this infection. This study was undertaken to determine the Prevalence of dengue cases in Kannur district from October 2016 to April 2017 by IgM Capture ELISA method and to correlate certain haematological and biochemical parameters in dengue patients. A total of 380 clinically suspected dengue patients who presented to the hospital for routine dengue testing were taken for the study. Among the 380 clinically suspected dengue patients, 65 samples were tested positive for dengue viral infection. Majority of the dengue patients were males. Most of the dengue patients belong to the age group 21-40 years (55%). Among the dengue confirmed patients, 57%(37) of patients showed platelet count below 100000/cu.mm and had severe thrombocytopenia. Out of the 65 dengue positive cases, leucopenia were evident in 31 (48%) patients. About 42 (65%) cases and 31 (48%) cases showed increased AST and ALT level respectively.

Keywords: Dengue, Hepatomegaly, AST and ALT, Thrombocytopenia.

Introduction

Dengue fever is a mosquito-borne virus disease of humans. DENV is a member of the Flaviviridae family and is grouped within the flavivirus genus together with other pathogenic viruses including West Nile virus (WNV), Japanese encephalitis virus (JEV), tick-borne encephalitis virus (TBEV) and yellow fever virus (YFV). Flaviviruses belong to the family of flaviviridae, which have 70 different antigenically related members. Most of the flaviviruses are arboviruses (arthropod-borne viruses) ⁽¹⁾

There are four distinct dengue virus (DENV) serotypes that share antigenic relationships (DENV-1, DENV-2, DENV-3, and DENV-4), and although infection with one serotype confers lifelong protection against that serotype, it does not necessarily protect against a secondary infection with a heterologous serotype. The name

dengue originated from the Swahili word for “bone – breaking fever “or the word for “the walk of a dandie” in Spanish ⁽²⁾. The exact clinical and laboratory profile is crucial for diagnosis as well as successful management of the patients. Currently, the most commonly used methods for dengue diagnosis include detection of: i) virus by cell culture; ii) viral nucleic acid; iii) DENV antigens or specific antibodies raised to them. Using a combination of two or more of these techniques increases the accuracy of diagnosis. ⁽³⁾ Dengue infection is an emerging disease and is a major health problem in our country. Globally the incidence of dengue has increased in the recent years. The WHO estimates that presently about two fifths of the world population is at risk for this viral infection. ⁽⁴⁾ Dengue fever was first reported by Benjamin Rush in 1780 as “break bone fever.” It is a mosquito borne viral infection with

four serotypes causing severe dengue fever, dengue with warning signs, and dengue without warning signs.⁽⁵⁾ Severity of the illness is determined by various risk factors such as age, pre-existing illness, infecting serotype, and secondary infection. A second infection with a different serotype leads to more severe form of the disease than the primary infection.⁽⁶⁾ The first major wide spread epidemics of DHF/DSS (Dengue Hemorrhagic Febr/Dengue Shock Syndrome) occurred in India in 1996 involving areas around Delhi and Lucknow and then it spread to all over the country.⁽⁷⁾

In the last decade, dengue has assumed pan-India proportions. Outbreaks and deaths have been reported from northern states of Haryana, Punjab and Uttar Pradesh; southern states of Andhra Pradesh, Tamil Nadu and Karnataka; western states of Gujarat and Rajasthan; and eastern state of West Bengal. In fact, the case fatality rate has been above 1% over the last 10 years.⁽⁸⁾ DENV is transmitted by mosquito bites that make the human body's skin the entry route of the virus. The uncommon modes of transmission are identified as vertical transmission from mother to foetus, transfusion-related transmission, transplantation related transmission, and needle-stick related transmission. Precise laboratory diagnosis of dengue infection is important not only for appropriate specialist clinical care but also for accurate public health surveillance. Following onset of infection in patients, fever develops in synchrony with viraemia, when virus particles are found in plasma or serum, blood cells and tissues of the immune system (spleen, liver, lymph nodes, and bone marrow). Various methods have been developed to analyze the antigen/antibody responses during dengue infection. In comparison to other techniques available, these are generally less expensive, easier to perform and, notably, can discriminate between primary and secondary infection.

The Armed Forces Research Institute of Medical Sciences (AFRIMS) developed an IgM antibody-capture enzyme-linked immune sorbent assay (MAC-ELISA) for dengue in regions where dengue and Japanese encephalitis virus co-circulate. Dengue-specific IgM in the test serum is detected by first capturing all IgM using human-specific IgM bound to a solid phase. The assay uses a mixture of four dengue antigens (usually derived from dengue virus-infected cell culture

supernatants or infected suckling mouse brain preparations).⁽⁹⁾ Compared to the haemagglutination inhibition assay as the gold standard, MAC-ELISA shows a sensitivity and specificity of 90% and 98%, respectively.

Standard haematological parameters such as platelet count and haematocrit are important and are part of the biological diagnosis of dengue infection. Therefore, they should be closely monitored. Thrombocytopenia, a drop in platelet count below 100000 per μl , may be occasionally observed in dengue fever but is a constant feature in DHF. Thrombocytopenia is usually found between the third and eighth day of illness often before or simultaneously with changes in haematocrit. Thrombocytopenia has always been one of the criteria used by WHO guidelines as a potential indicator of clinical severity. In the most recent 2009 WHO guidelines, the definitions generally describe a rapid decline in platelet count or a platelet count less than 150,000 per micro liter of blood. In fact, previous studies reported DHF in adults without shock, in which platelet counts mildly to moderately decrease on the 3rd day until the 7th day of illness and reached normal levels on the 8th or 9th day.^(3,10-12)

Liver is one of the major target organs involved in acute dengue virus infections; hepatomegaly and increased levels of AST and ALT are common manifestations of this infection. In dengue virus-induced hepatitis, the level of AST has been found to be higher than the level of ALT. This differs from the pattern in viral hepatitis but is similar to that seen in alcoholic hepatitis. The exact cause of this is uncertain, but it has been suggested that it may be due to excess release of AST from damaged monocytes during dengue infection.⁽¹³⁾

The pathogenesis of Dengue infection depends on the cell and tissue tropism of DENV, usually infects peripheral blood mononuclear cells within a few days of the infective mosquito bite. Detectable levels of anti-dengue antibodies appear 5-6 days after fever. Detection of IgM antibodies to dengue virus by ELISA is a valuable procedure, particularly in second and subsequent infections where the occurrence of complications is high. Dengue IgM ELISA is for the qualitative detection of IgM antibodies to dengue antigen in serum as an aid in the clinical laboratory diagnosis of patients with clinical symptoms consistent with dengue fever. Hence the study was undertaken to determine prevalence of dengue cases in Kannur

district, Kerala by IgM Capture ELISA method and to correlate certain haematological and biochemical parameters in dengue patients.

The samples are collected from patients with fever, headache and myalgia. Patients of all age are considered in this study, necessary data of patients which includes the clinical history, signs and symptoms of patients and lab results of patients are collected. For all patients Dengue IgM ELISA is performed and those with positive results are further studied for other lab investigations such as Platelet count, Total count, ALT, and AST estimation.

A total of 380 samples are collected from suspected cases of dengue patients and is used for laboratory examinations of dengue fever. The samples are tested serologically by Dengue IgM Capture ELISA. Patients included in this study will undergo routine hematological investigations like platelet count and total count. Complete Blood cell Count (CBC) is carried out in auto cell analyzer. Blood smear was stained with Leishman's stain, and a differential count performed to determine the percentage of lymphocyte, neutrophil, eosinophils, and monocytes. Platelet count is recorded in each individual. The biochemical investigations includes the estimation of ALT and AST level for liver involvement. These tests are performed on automated analyzer.

Among the 380 clinically suspected dengue patients, 65 samples were tested positive for dengue viral infection and 315 samples were seronegative for dengue. The intensity of the yellow colour on the micro titre well was read at the wavelength of 450 nm with a reference filter of 600-650 nm. Positive samples produce yellow colour after the addition of stop solution. Among the 380 clinically suspected dengue patients, 65 (17%) samples were tested seropositive and 315 (83%) samples were seronegative for dengue IgM ELISA. Table 1 shows sex distribution of dengue suspected patients. The age range from 6 years to 90 years with maximum cases of fever occurring in the age group of greater of 21 – 40 years(55%). Table 2 depicts the age distribution of dengue confirmed patients. The age group distributions was 0- 20 years (9%), 21-40 years (55%) 41- 60 years (29%), and above 60 years (6%) in overall clinically suspected dengue fever cases. Out of 65 positive samples, 44 were males (68%) and remaining 21 (32%) were females, majority of the

dengue patients were males. Most of the dengue patients belong to the age group 21-40 years (55%) followed by 41-60 (29%), 0-20 years (9%), and >60 (6%).

Platelet count of all the dengue suspected patients were noted. Among the dengue confirmed patients, 37(57%) of patients showed platelet count below 100000/cu.mm and had severe thrombocytopenia. In 14% of patients it was observed that platelet count in between 100000 to 150000cu.mm and 29% patients showed >150000. Out of the 65 dengue positive cases, leucopenia were evident in 31 (48%) patients. Leucopenia was more evident in patients with acute stage of illness. AST and ALT levels of all the dengue confirmed patients are noted. About 42 (65%) cases and 31 (48%) cases showed increased AST and ALT level respectively. The present study also revealed that 35% cases (23) of AST and 32% cases (21) of ALT level are within the normal range. But 20% cases (13) patients showed ALT level >500.

Due to climate changing urbanization, poor living conditions and inadequate waste management, vector borne diseases like dengue fever and chikungunya virus are more common. Although vector control programs are launched in endemic countries every year, yet dengue fever has become a tropical country provides suitable weather for Aedes mosquito to grow and an increase in the disease burden has been noticed in recent years. Vector control is known to be a good method for prevention of vector borne diseases.

The current study has shown a male predominance (68%) as compared to the females (32%). Male predominance correlates with majority of studies. Study reports from Asian countries such as Cambodia, Malaysia, Sri Lanka, Singapore and Philippines and India reveal the high preponderance of males in dengue fever and pointing out the fact that it may be due to the outdoor work habit of men compared to females. A majority of the studies points out the fact that males have more outdoor work habits compared to females. They are more exposed to the mosquito bite during their day time work or while travelling to the work site in the early morning or returning from the work site in the late evening. So the high number of infected dengue cases in adult age groups and in males is strongly indicating the involvement of work component in this infection because children and women are spending much

time in indoors and less exposed to the vectors of dengue infection.

In this study it was observed that the mean age group affected was 21 - 40 years. Most of the dengue studies done in India shows younger population predominantly involved(Pruthvi *et al.*, 2012).⁽¹⁴⁾ This predominance of adults may be due to involvement of adult groups in outdoor works compared to low age group as they have the more chances of exposing infected mosquitoes than the low age groups. Present study also revealed , the common haematological findings were thrombocytopenia, leucopenia, and elevated transaminase level. The peripheral smears showed leucopenia. The serum levels of AST and ALT were also raised in a substantial proportion of cases. The incidence of severe thrombocytopenia in this study where 57%. In a study performed by Ahmed *et al.*, (2008) with 35 patients revealed that 86 % of the dengue patients had thrombocytopenia.⁽¹⁵⁾

The reduction in platelet count was attributed to depression of the bone marrow, direct infection of the megakaryocytes by virus and the presence of antibodies to platelets. In the review article by Lei *et al.*, thrombocytopenia was attributed to autoantibodies which induce platelet lysis via complement activation. The study also reported the cross reactivity of antibodies directed towards dengue virus proteins and platelets suggesting the pathogenic role of anti-platelet auto-antibodies during dengue virus infection.⁽¹⁶⁾

Low leucocytes count in dengue fever may be due to virus induced inhibition/destruction of myeloid progenitor cells. Current study it was found that 31 (48%) cases had leucocytes count below 4000/cu mm. Low *et al.*, (2011) observed that leucopenia was more marked in dengue patients with increasing age and they discussed the usefulness of leucopenia in aiding early clinical diagnosis of dengue.⁽¹⁷⁾ Leucopenia was observed in 26% of patients by Ratagiri *et al.*⁽¹⁸⁾

In our study it was observed that, liver amino transferase level showed increased level. ALT level were elevated in 68% of the patients where as AST level were elevated in 65%. There for elevation of ALT occurred in most cases either together with AST elevation or as alone alteration. Similar observations was found in Pakistan, study conducted by Ahmed SI *et al* raised ALT levels were noted in 62% patients and raised AST levels

were noted in 51% dengue fever patients.⁽¹⁹⁾

Dengue IgM Capture ELISA is used for early and accurate diagnosis of acute phase of illness and is a qualitative detection of IgM antibodies to dengue antigen in serum. The dengue IgM Capture ELISA should be used in conjunction with other dengue serology, haematological and biochemical parameters can save a life rather than waiting for symptom to progress and deteriorate. Hemo concentration, leucopenia, thrombocytopenia, raised AST, raised ALT and plasma cytoid lymphocytes in peripheral blood smear shall give enough clues to test for Dengue serology so as to reduce the morbidity and mortality due to this disease.

Strict monitoring of clinical condition and haematology parameters is required to prevent complication which makes early diagnosis pertinent. Early diagnosis is also vital for exclusion, as dengue fever is most of the cases in clinically indistinguishable from other febrile illness prevailing in “dengue season.” Furthermore, early diagnosis plays a crucial role in forecasting a timely warning of an epidemic and in undertaking effective vector control measures. In this study it is concluded that the incidence of dengue fever in Kannur district, Kerala during the period from October 2016 to April 2017 was 17%.

TABLES

Table 1: Sex distribution of dengue suspected patients

Sex	No. of patients	Dengue seropositive	Dengue seronegative
Male	212	44	168
Female	168	21	147
Total	380	65	315

Table 2: Age distribution of dengue confirmed patients

Age group	No. of patients	Percentage
0 – 20	6	9%
21 – 40	36	55%

41 – 60	19	29%
>60	4	6%

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