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Original Research Article

Sensitive and Specific Markers for Detection of Dengue Virus Infection from Suspected Patients in Sivagiri Taluk, Tamil Nadu

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ABSTRACT

Background: Dengue viral infection is one of the most important public health problems in India. Therefore, early diagnosis of dengue infection during the febrile stage is better to understand for the adjusting appropriate management. This study is to identify the specific markers of clinical features in the acute stage of dengue infection during a major outbreak of dengue virus that is also confirmed by immunological assays. The study was performed to investigate the various dengue suspected cases during first week of June 2014 to August 2015. During this period, many suspected cases of Dengue fever (DF) were reported from rural and urban areas of Sivagiri Taluk, Tamil Nadu.
Results: A total of 269 blood samples were collected from dengue suspected cases and were tested for dengue specific immunoglobulin M (IgM) and IgG antibodies. The suspected dengue cases consist of 29 children (≤ 12 years) and 240 adults. Among the 269 suspected cases, 5 (1.86%) children and 27 (10.04%) adults were laboratory confirmed. A total of 32 cases were positive by both dengue IgM capture assay and Dengue IgG/IgM rapid test kit (n = 21). Based on the clinical features for predicting laboratory confirmed dengue infection, the sensitivities of fever, diarrhea, vomiting, rashes in skin, myalgia and petechiae are 85.18%, 88.88%, 70.58%, 84.61%, 81.25% and 73.68%, while the specificities for above features are 97.13%, 97.53%, 96.34%, 97.53%, 96.73% and 97.13%, respectively.
Conclusion: Specific and sensitive immunological assessments would be the useful predictive markers for early diagnosis of dengue infection during a large outbreak in the infection.

Keywords: Dengue virus; early diagnosis; clinical features; IgM/IgG antibodies and predictive markers.

INTRODUCTION

Dengue virus (DENV) is a mosquito-borne flavivirus and transmitted by Aedes aegypti and Aedes albopictus. It is widely distributed throughout the tropical and subtropical countries especially urban and semi urban areas, and causes up to 100 million infections annually. DENV causes dengue hemorrhagic fever (DHF) by hemorrhagic signs in infected cells and is also causes dengue shock syndrome (DSS) and is characterized by features of DHF plus evidence of circulatory failure manifested by hypotension or hypertension, cold clammy skin and restlessness. [1] Dengue fever (DF) and DHF are caused by four antigenically distinct serotypes (DEN-1, DEN-2, DEN-3 and DEN-4) of dengue viruses. The four distinct serotypes have recently undergone a dramatic expansion in range, affecting tropical and sub-tropical regions around the world, and the global incidence of DF and DHF has grown dramatically in recent decades.

DENV is detected by means of several biological tests that its routine use in clinical diagnosis, Because IgM does not become detectable until 5-10 days after the onset of illness in secondary infection. [2] Immunoglobulin G (IgG) antibodies against DENV are detectable after 10-14 days and remain for life time. [3] The efficiency of strategy is estimated at >93% only. Therefore, early diagnosis of dengue infection during the febrile stage is useful for the rapid identification risk areas using serological MAC-ELISA. [2] Therefore, the present study was undertaken to identify a specific marker for the diagnosis of early stage of dengue infection during the outbreak in Sivagiri Taluk and to identify dengue patients using antigen capture ELISA technique and Dengue IgG/IgM rapid test device.

MATERIALS AND METHODS

Study area

The study area (146 sq. km) lies between 8° 27' 11" N-18° 20' 26" N latitudes and 74° 16' 16" E-80° 11' 11"E longitudes. This area receives rainfall, i.e., Southwest (June -September) and Northeast (October-December) monsoon. The rainfall is moderate to high, with an average of 900 mm to 1050 mm annually. The mean annual temperature is 26°C -28.5°C. The total population of the study area is 98,697 (Source: Census of India 2011). The literacy rate is 86%. A network of government-run Primary Health Centers (PHCs) in rural areas and General Hospitals in urban areas serves the health needs of the people. Therefore, the region has been chosen for the present work to identify and compare the clinical features between dengue positive and negative cases.

Sampling design

Based on the occurrence of mosquito breeding sites and the level of infection caused by DENV, suspected cases are identified as dengue infection risk area and non-risk area. The availability of breeding sites is to favor the increase of the population of dengue vector Ae. aegypti as well as Ae. albopictus. Therefore, the selected site was divided into 2 × 2 km grids, so that each grid would contain at least one village (rural) or ward (urban). A total of 35 sites were surveyed from the study area on a systematic random basis. This study was approved by the Institutional Ethical Committee and the committee also approved to use the stored blood samples.

Sample collection

Blood samples were collected from dengue suspected patients who were likely to have DF symptoms and the corresponding clinical data were also noted through pre-designed structured questionnaire. A total of 269 DF patients' blood samples and their severity were collected from selected sites during June 2014 to August 2015 by practiced staff nurse. These samples were collected within 3 to 7 days after onset of symptoms and stored at -20°C. Most suspected samples only were directly tested with the rapid visual test for detection of Dengue NS1 Antigen and differential detection of IgM & IgG antibodies (J. Mitra & Co. PVT. Ltd) and other sera were tested and studied in laboratory against DENV, using the antigen capture ELISA technique.

Screening the blood samples

The blood samples of each site were screened against DENV using antigen capture ELISA and Dengue IgG/IgM rapid test device (J Mitra & Co Pvt. Ltd, Delhi). The procedure for antigen capture ELISA was carried out as previously described, with modification. [4] In brief, micro well plates were coated with polyclonal antibodies (100µl/well) overnight at 4°C, and then the plates were incubated with a blocking reagent. After removal of the blocking solution, a series of diluted 100 µl/well samples was added and incubated for 1 h at 37°C. After the plates were washed, 100 µl/well of diluted HRP-conjugated MAb was added and incubated for 30 min at 37°C. After further washing, 100µl/well of TMB solution was added, and the reaction was stopped after incubation for 10 min with 100 µl/well of 1 N sulfuric acid

and absorbance was read at 450 nm in a micro plate reader. The Dengue IgG/IgM rapid test devices were performed according to the manufacturer's instructions. For the prospective study, only acute blood samples were tested with the kit in laboratory as well as at the place of visit. The sensitivity and specificity of the items for predicting dengue infection were determined for each assigned cut-off value.

RESULTS AND DISCUSSION

In this study, the blood samples were subjected for DENV detection by antigen capture ELISA. Samples were considered as dengue positive if their optimum density values were greater than negative control values. In other hand, a result was considered as positive for dengue when the optical density was higher than 0.1 for the DENV IgM and when the OD of the anti-DENV ELISA was higher than the OD of the anti-JEV ELISA. A total of 269 blood samples with clinically suspected dengue cases were screened against DENV and 32 were detected as dengue positive and 237 as dengue negative from the selected 35 sites and the results are given in table 1.

Table 1: Risk status of the suspected and confirmed dengue cases by MAC ELISA in Sivagiri Taluk

Status	No. of sites selected	No. of sites positive for DENV	No. of individuals tested		
			Positive	Negative	Total
Non-risk area	20	1	4	99	103
Risk area	15	12	28	138	166
Total	**35**	**13**	**32**	**237**	**269**

Male was predominant (65.2%) among the dengue cases. A total of 103 samples tested from non-risk area were negative for DENV and out of 166 samples which were tested from risk area, a total of 32 samples were recorded DENV positive from the risk and non-risk areas. These results agreed with commercially available dengue specific kits are specifically confirmed that showed positive result to the dengue virus. These results were exactly matching the results which were detected using the commercially available dengue specific kits which confirmed the positive results of DENV. [5]

Table 2: Detection of primary and secondary immune status during the DENV infections in Sivagiri Taluk by Dengue IgG/IgM rapid test device

Status	No. of sites selected	No. of sites positive for DENV	No. of individual tested				
			IgM positive	IgG positive	IgM/IgG positive	Negative	Total
Non-risk area	20	2	2	1	-	22	25
Risk area	15	9	11	16	2	6	35
Total	**35**	**11**	**13**	**17**	**2**	**28**	**60**

Table 3: Value of selected clinical features in predicting a laboratory diagnosis of DENV in suspected patients

Clinical features	Dengue Positive (N=32)	Dengue Negative (N=237)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Fever	23	7	85.18	97.13	76.66	98.34
Headache	19	8	7916	96.73	70.37	97.93
Diarrhea	16	6	88.88	97.53	72.72	99.16
Itching skin	10	5	76.92	97.93	66.66	98.75
Nausea/Vomiting	12	9	70.58	96.34	57.14	97.93
Skin rash	11	6	84.61	97.53	64.70	99.16
Myalgia	13	8	81.25	96.73	61.90	98.75
Petechiae	14	7	73.68	97.13	66.66	97.93

The determination of predictive values was performed based on the clinical/laboratory features for predicting laboratory-confirmed dengue infection. Adult patients have higher incidences of fever, myalgia, headache and abdominal pain. The sensitivities of fever, headache, myalgia, and rashes in skin were 85.18%, 79.16%, 81.25% and 84.61%, while the specificities for above features were

97.13%, 96.73%,96.73% and 97.53%, respectively. Fever, head ache and diarrhea

The most frequent clinical features in dengue patients were fever (76.66%), myalgia (61.90%), and headache (72.72%). However, these features were also very prevalent among dengue negative patients and were useless for discriminating between dengue positive and negative patients. From clinical history, only taste disorder (detection of IgG; IgM and/or both) and the report of rash (ELISA test) were associated with dengue diagnosis (Table 2). However, rashes appear usually late in clinical course of dengue infection and was not a good predictor of acute dengue infection. Therefore, present study revealed the several clinical features as predictors of laboratory-confirmed dengue infections. No single laboratory test was good as enough in terms of positive predictive value.

However, we found that the positive predictive value for combination of other clinical features and negative predictive value is 97.4%. From this result, simple clinical and laboratory based specific markers can serve as an adjuvant in addition to history and physical examination, and also reduce the possible cost for universal laboratory diagnostic screening. [6,7] The incidence of some elevated blood parameter levels and prevalence of DHF in adults were also higher than children. [8] The symptom combinations identified here while having high positive predictive value still had low sensitivity. Therefore it may be a useful addition to the clinical evaluation and there is still a need to identify tests with better sensitivity and specificity. Besides, the accuracy of dengue diagnostic tests depends on the prevalence of dengue and time of sampling. [9] According to WHO, low specificity of clinical features were explored and the low accuracy rate was then confirmed by the laboratory. [10] The difficulty to identify early clinical predictors of dengue infection in adults has been described [11] in a large study. Although, they identified eye pain, diarrhea and absence of upper respiratory as the had high positive predictive value 76.66%, 72.72% and 70.37%, respectively (Table 3). symptoms, they are independently associated to confirmed dengue cases, they also highlighted the low predictive value of these features, alone or in combination, for early infection in adults. In spite of this relatively low accuracy, the use of management protocols based on clinical diagnostic scales proved useful to reduce hospitalizations due to dengue. The interpretation and generalization of the results of studies such as this one must consider the fact that diagnostic accuracy of clinical manifestations also depends on their frequency in the dengue negative patients. For instance, low platelet counts may be useless to discriminate dengue from other febrile illnesses in a place where malaria is endemic, as low platelet counts were also frequent in the later. [6] In our study, laboratory tests and clinical features were required as indicated by clinical doubt and we are unable to describe the prevalence of other diagnoses. However, through this attempt, it can be possible to identify the positive cases from the potential risk area with any of the existing tools. Even though, these clinical and laboratory findings may serve as predictive markers to promote early diagnosis of dengue infection. Therefore, the specific clinical marker is an alternative tool for epidemiological surveillance of DENV infections.

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