



Significance of Sequential Emergence of Serological Responses by Dengue Patients as Community Level Sero-Epidemiological Indicators of Disease Progression: A Study in Barmer, Rajasthan, India

KEYWORDS

Dengue, sequential emergence, IgM, NS1

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ABSTRACT *Dengue & Dengue Haemorrhagic Fever have emerged as major public health problem in India. A prolonged outbreak of DF & DHF has been reported from Barmer, Rajasthan. Sequence in emergence of serological parameters, NS1 viral protein and IgM antibodies by the dengue patients is the focus in the present study. As outbreak progressed the proportion of cases carrying NS1 antigen decreased and IgM antibodies increased from the month of August to December, 2013. The descending trend of NS1 protein and ascending trend of IgM antibodies coincided with reduction in disease load as only 31 cases were recorded till 15th December. We infer that extrinsic stock of virus is not infecting a new case in a serially progressing outbreak but intrinsic virus (Man to man), which is only antigenically sufficient to induce IgM antibodies and insufficient to remain in any more existence as the NS1 antigen is circulating across the population.*

INTRODUCTION

Dengue Fever (DF) and Dengue Hemorrhagic Fever (DHF) has emerged as major public health problem in India (75454 cases & 167 deaths during 2013). About 4413 cases and 4 deaths have been reported officially from north western desert state Rajasthan, India¹. An outbreak of DF & DHF extending from January till December, 2013 has been reported from Balotra town, Barmer district, Rajasthan, India (898 cases). Containment of the persistent transmission of dengue within this small town provided us an opportunity to study as to how in a progressive disease outbreak the human host respond serologically against the ongoing viral antigen and that can prevailing serological indices (IgM, IgG and NS1) as observed in the patients at a given point of time could be used as the community indicators of age, severity and prospective continuation of disease?

We hypothesize that once disease cycle is established in an area through infective mosquito bites to the susceptible human hosts, generation of new cases is the result of horizontal transmission of virus, travelling linearly across vectors and susceptible human host systems within the area of vector's flight range and human movements in a geographical area for a given segment of time. Immunological response of a new case against this transiting virus depends directly on the quality and quantity of viral inputs transferred into the case either horizontally (from an infected person) or vertically by an extrinsic virus (from virus passed through mosquito generations). It has been observed that during initial phase of disease eruption, diagnosis of dengue cases become a difficult task as pathogen specific IgM antibodies do not appear in the patients for about 7-10 days². Nevertheless, disease burden in the endemic settings keep on increasing due to addition of new cases. We hypothesized that type of the serological response by the patients at a given point of time (test positivity for NS1, IgM) in a progressive outbreak may speak as the community level indicators of strength of pathogen being transmitted and as such may serve as the sero-epidemiological marker of how old is an outbreak and that what could be the possible number of transmission competent hosts at given point of time in an ongoing outbreak. We further hypothesize that while presence

of response like NS1 may indicate prospective speedy spread of disease for viral protein being available for man to man transfer, on other hand, more positivity of IgM like parameters may strengthen expectation of relatively slower and controlled progression of disease in the time to come. A study of sequence in appearance of serological parameters therefore could serve as the community level indicators of an ongoing outbreak in addition to being the individual parameters of diagnosis. In present paper, we report the results of emergence of serological parameters conducted on a year long outbreak of dengue at in Balotra, Rajasthan, India.

METHODS**Study Area**

Study area, Balotra, is situated in the central part of north-west boarder district Barmer, Rajasthan, India. The town represents a typical desert ecosystem characterized by sparse vegetation, dry climate and scarce rain fall. Balotra town has population of about 95, 513 as per the 2001 census and is spread in an area of 11.20 sq. km.

Outbreak Investigation and serological tests data analysis

Emergence of cases of dengue started from Balotra, Barmer, Rajasthan, India since January, 2013. As a Reference Virological laboratory and as one of the surveillance centres of National Sentinel Surveillance network in Rajasthan for vector borne diseases as identified by the National Vector Borne Disease Control Programme (NVBDCP) Government of India, Desert Medicine Research Centre (DMRC), ICMR (Indian Council of Medical Research), was requested to investigate the outbreak situation and provide the diagnostic support for conducting Mac-ELISA test on the serum samples tested positive for NS1, IgM & IgG by the local hospital laboratory. To address the task of investigating outbreak, detailed analysis of the data on serological test results (NS1, IgG & IgM) performed by local hospital, Balotra since January till December, 2013 was made.

Mac-ELISA tests on serum samples

Mac-ELISA was performed on the serum samples referred by local hospital, Balotra, Rajasthan, using the test kits developed and supplied by the National Institute of Virology, Pune, India.

RESULTS

Table 1 shows percentages of appearance of serological parameters viz; NS1 protein, IgM and IgG antibodies in dengue patients during ongoing outbreak. In the month of January, 2013 only sporadic cases of dengue were reported whereas in February and March, no case was recorded.

The active and continuous outbreak of dengue started from the month of August, when 19 cases were reported to local hospital. The data show that during this period 94.7 % of the cases carried NS1 antigen and only 10.5 % cases showed IgM antibodies. This proportion of NS1 and IgM antibodies resulted to subsequent high magnitude of 228 cases in the subsequent month of September, 2013. Of these 228 cases, 221 cases (96.9 %) carried NS1 antigen while only 5.2% cases showed IgM antibodies (Figure 1& 2).

In succeeding month of October, 2013, as many as 328 cases of dengue were reported of which 268 (81.7 %) carried NS1 antigen and 56 (17.0 %) carrying IgM antibodies. Although in the month of October, 2013, incidence of dengue reached its peak yet the proportion of patients carrying IgM antibodies also increased from 5.2% in the month of September to 17.0 % in the month of October, 2013 (Figure 1& 2).

In the month of November, 2013, 222 cases of dengue were reported which showed NS1 protein in 90 (40.5%) cases whereas, IgM antibodies in 152 (68.4%) cases. Proportion of patients carrying dengue antigen decreased from 81.7% in month of October to only 40.5 % in the month of November. On contrary, IgM antibodies increased from 17.0% in the month of October to 68.4% in month of November, 2013. The descending trend of NS1 protein and ascending one of IgM antibodies in human patients coincided with a corresponding reduction in the disease load in subsequent month when only 31 cases of dengue were recorded till December, 15, 2013 (Figure 1& 2).

DISCUSSION

In north-western district, Barmer, Rajasthan, India, a major outbreak of dengue has been reported in its Balotra town. There exists only one Government hospital in the town and our survey of the town showed that all the cases occurred in the town were reported to the hospital. As per local hospital records, 898 cases have been reported from January till 15th December, 2013. In present paper a hospital based study has been undertaken on the serological test results of the dengue cases.

The analysis of the observations showed that serological response of the infected human hosts in horizontal passage of virus through vector mediated transmission across community, had followed a definite sequence. The virus's travel from one infected human to other healthy host, via mosquito systems, has caused different but sequential expression of serological response of human hosts not only at individual level but at community level also. It has been observed that early batches of the patients showed more presence of NS1 antigen whereas later cases showed more number of IgM antibodies. It is understandable in an individual human host system that initial inoculation of virus causes appearance of NS1 antigen first and when infection 5-8 days old, IgM antibodies appear.³ But at community level, where every new case represents a new host system for virus, how NS1 antigen did not appear in new patients testing dengue positive after almost 3 months of outbreak? Our data show that in the month of November, 2013, only 40.5% of dengue patients showed NS1 antigen whereas more than 68.4% of patients were positive for IgM antibodies. We infer that this may be due to the fact that extrinsic stock of virus is not infecting a new case in a serially progressing outbreak but the intrinsic virus (Man to man), which is only antigenically sufficient to induce IgM antibodies and insufficient to remain in any more existence as the NS1 antigen in host blood, is circulating across the population affected by the disease outbreak. Our

inferences are supported by earlier work which reports that only 10^5 units of virus per ml of human blood are available in a patient⁴. Keeping this in view it appears that in addition to serve as individual sero-diagnostic parameters of dengue patients, these immunological responses (NS1, IgM) could be the community or public health indicators of extent of viraemia circulating during an outbreak at a given point of time.

It has been reported by World Health Organization that in individual patients of dengue NS1 protein is expected to appear till 5 days of infection followed by host response in the form of IgM antibodies³. We report that horizontal (man to man) transmission of dengue virus weakens viral protein during progression of disease in an area and appearance of NS1 or IgM parameters can serve as community level indicators of aggression of infection in a setting. Availability of such indicators could influence the outbreak control measures to pin point the points of intervention in an ongoing dengue outbreak.

There have been few pioneer studies on the host response of the dengue patients in terms of Th1 response stimulating CTL activity during initial phase of infection and Th2 response in later stages.⁵ Association of high levels of IL-12 with less severe cases of dengue has also been reported.⁶ In present paper, in addition to the individual parameters of diagnosis, we report on the epidemiological significance of serological responses to develop indicators of community level progression of the disease. The observations reported could be useful for the public health understanding of dengue outbreak.

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Table 1. Community level Sequential emergence of serological responses in the patients of Dengue Fever

S.No.	Month, 2013	No. of samples tested	No. +ve for NS1	% +ve for NS1	No. +ve for IgM	% +ve for IgM	No. +ve for IgG	% +ve for IgG
1	January	4	3	75.0	2	50.0	1	25.0
2	February	NC	NC	-	NC	-	NC	-
3	March	NC	NC	-	NC	-	NC	-
4	April	15	10	66.6	3	20.0	4	26.6
5	May	49	49	100.0	3	6.1	1	2.0
6	June	2	2	100.0	0	-	0	-
7	July	NC	NC	-	NC	-	NC	-
8	August	19	18	94.7	2	10.5	0	-
9	September	228	221	96.9	12	5.2	19	8.3
10	October	328	268	81.7	56	17.0	93	28.3
11	November	222	90	40.5	152	68.4	171	77.0
12	December	31	10	32.2	15	48.3	25	80.6
	Total	898	671	74.7	245	27.2	314	34.9

* Data up to 15th December, 2013. (NC= No case)

Figure 1. Sketch showing sequential serological responses of dengue patients during ongoing outbreak.

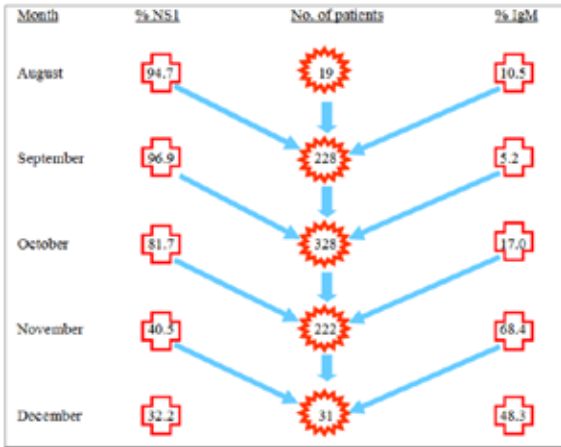
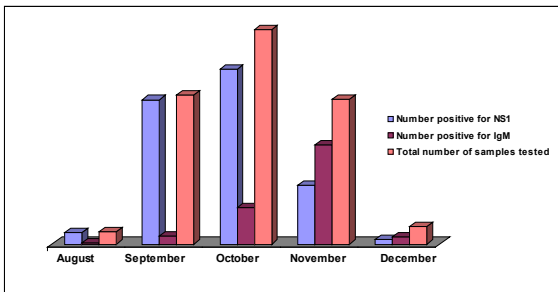


Figure 2. Sequence in progression of NS1 antigen and IgM antibodies in dengue patients (August-October, 2013)



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