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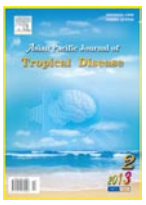

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Retrospective analysis of dengue specific IgM reactive serum samples

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PEER REVIEW

Peer reviewer

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Comments

This is a good study in which the authors have clearly shown that secondary infection in dengue is highly prevalent in Kolkata city.
(Details on Page 145)

ABSTRACT

Objective: To conduct a retrospective analysis of dengue cases in Kolkata, on the basis of presence of anti-dengue IgM in their sera and presence or absence of anti-dengue IgG and dengue specific Non structural 1 (NS1) antigen in each of the serum sample. **Methods:** Sample was tested quantitatively employing ELISA technique, using Biorad test kits, with a view to get a more comprehensive picture of dengue in an urban endemic area and also to evaluate individual cases. **Results:** This reconstructed study revealed that of those 91 dengue cases, 70.3% (64) and 29.7% (27) were suffering from secondary and primary dengue respectively, showing that number of secondary dengue cases were much more than that of primary dengue cases with a possibility of emergence of DHF. A small proportion of cases 18.7% (17) were reactive for NS1. The duration of fever in NS1 antigen positive cases varied between 5 and 7 days. Of 17 NS1 reactive cases, 10 (10.9%) and 7 (7.7%) were suffering from secondary and primary dengue respectively. **Conclusions:** Early detection of primary and secondary dengue cases would be facilitated by utilizing all three parameters (NS1 antigen, anti-dengue IgM and IgG) helping to evaluate, monitor and treat a dengue case effectively.

KEYWORDS

Dengue, Specific IgM, Serum sample

1. Introduction

Retrospective analysis of dengue specific IgM reactive serum samples of 91 cases originated in Kolkata was performed. Presence or absence of dengue specific non structural 1 (NS1) antigen and IgG antibodies in each serum sample was noted. The aims were to find out proportion of IgM reactive people positive for NS1 antigento determine proportion of primary and secondary dengue cases[1,2], and to evaluate role of three tests when done together to demonstrate the status of a patient in laboratory diagnosis of dengue[3,4].

In a dengue patient, detection of only dengue specific IgM antibodies provides only a partial information, denoting that the patient is suffering from dengue, at least 5 days after the onset of fever. So the patient cannot be diagnosed at an early stage of the disease and also it cannot be classified whether he is suffering from primary or secondary dengue infection, which information is important for effective treatment or monitoring. Hence the rationality of this study is to find out the proportion of primary or secondary cases among only IgM reactive cases, which could not have been diagnosed at early stage and also the duration of the fever in such patients. The motivation is to project the utility of both dengue specific antigen and antibody (IgM and IgG) tests simultaneously in laboratory practice, helpful on the part of both the physician and patient. In some studies for serological diagnosis of

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dengue in laboratory practice, only dengue specific IgM and IgG antibodies have been utilized (owing to non-availability of NS1 antigen at that time), and in some other only NS1 or MAC-Elisa and NS1 antigen have been tested, not providing unfortunately all the required information on dengue fever cases as hypothesized in the present study. Hence the precise objective of the study is to get complete information about those 91 IgM reactive patients related to the duration of the disease and categorization of primary and secondary dengue cases by testing retrospectively dengue specific NS1 antigen and IgG antibodies of all these serum samples.

2. Materials and methods

Dengue specific IgM reactive 91 serum samples diagnosed by the Department of Virology of the Calcutta School of Tropical Medicine between August and December 2010 were retrospectively analyzed quantitatively in June 2011, for presence or absence of dengue specific NS1 antigen and IgG antibodies. Bio-Rad test kits were used. Instruction of the manufacturer was followed. Necessary ethical approval has been taken (Approval No. VIHC/371 Dated 29.09.2009).

The dengue virus NS1 antigen test was defined as positive when the cut off value was greater than 0.535. IgG capture Elisa was assessed using patient's serum at 1/20 dilution. IgM/IgG ratio was used to demonstrate primary from secondary dengue virus infections. Dengue infection was diagnosed as primary, if the IgM/IgG ratio was greater than 1.4 (as we used patient's serum at 1/20 dilution). The infection was secondary, if the ratio was less than 1.4[1]. As in all those persons, MAC-ELISA was only studied, the blood had been taken from them at least 4 days after the onset of fever[1].

3. Results

Out of 91 dengue specific IgM reactive serum samples, analyzed retrospectively, dengue specific NS1 antigen was present in 17(18.7%) and IgG antibodies were detected in 64 (70.3%) samples. The duration of fever in NS1 antigen positive cases varied between 5 and 7 days. In this group, 77 patients (84.6%) were suffering from fever for 5-9 days. Out of 17 NS1 positive cases, IgG antibodies were reactive in 10 (10.9%) persons. Of 17 NS1 positive patients 11 persons were suffering from fever for 5 days, and 6 persons gave the history of fever varying from 6 to 7 d.

In this series, NS1 antigen positivity was found to be very low, amounting to only 18.7%. It demonstrates that for diagnosis of dengue Mac-ELISA technique is superior to NS1 antigen detection procedure when the history of fever is more than 4 days. Proportion of secondary dengue cases was significantly higher than that of primary dengue cases (Figure 1).

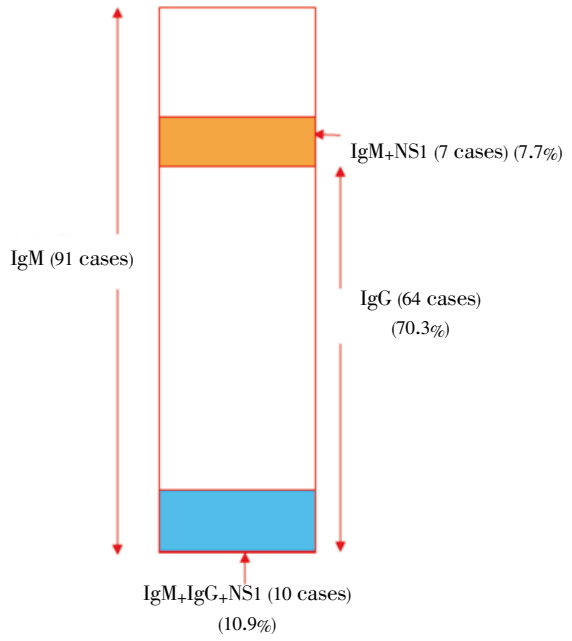

Figure 1. Retrospective analysis of 91 dengue specific IgM reactive patients categorizing the primary and secondary cases according to the duration of fever.

4. Discussion

NS1 antigen should usually be demonstrated from the very beginning of dengue fever. Here lies its importance. It will help immensely to monitor dengue from its early stage. On the other hand, IgM antibodies will appear in the blood of a dengue patient at least after 4 days of the onset of fever. By this time, the disease may have passed the critical stages as very quick onset of severe manifestations of dengue may occur in complicated cases. In such cases delayed diagnosis will not be so much helpful.

In this series 77 (84.6%) persons were suffering from fever for at least 5 to 9 days. In 17 NS1 dengue antigen positive cases the history of fever varied from 5 to 7 days. NS1 antigen should be found in the blood of dengue patients up to 9 days of the onset of fever[1].

Why then in a vast majority of dengue victims with the history of fever of 9 days NS1 antigen was not obtained? No clear cut answer can be put forward. Dengue virus NS1 antigen rapid test was used for onsite detection of imported dengue cases at airport[2]. For detection of dengue, NS1 protein was used in Vietnamese patients[3]. It was claimed that NS1 antigen in combination with MAC-ELISA on a single sample significantly improved the diagnostic algorithm without the requirement of patients' serum[4]. Our results show that dengue virus NS1 antigen detection alone is not a very reliable procedure for diagnosis of dengue fever cases. Absence of NS1 antigen in so many dengue confirmed cases in the present study with the history of fever for less than 10 d remains an enigma that requires a detail immunological study. Even when performed only with MAC-ELISA, improvement of

diagnostic algorithm seemed to be marginal demonstrating 17 cases only with the history of fever extending from 5–7 d. Based on IgM/IgG ratio, 91 dengue positive patients were further categorized into 27 (29.7%) primary (only IgM reactive) and 64 (70.3%) secondary dengue (both IgM and IgG reactive) cases. This result tallied with a previous longitudinal dengue serosurveillance study conducted in the same region[5,6].

According to the reconstructive study 91 dengue patients can be placed in four categories: (1) IgM reactive, IgG reactive, NS1 positive: 10 (10.9%) patients suffering from secondary dengue fever diagnosed between 5 and 9 days of the onset of fever; (2) IgM reactive and IgG reactive but NS1 negative: 54 (59.3%) secondary dengue patients diagnosed at least after 9 days of the onset of fever; (3) IgM reactive, IgG non-reactive but NS1 positive: 7 (7.7%) patients suffering from primary dengue, diagnosed between 5 and 9 days of the onset of fever; (4) IgM reactive, IgG non-reactive and NS1 negative 20 (22.01%) primary dengue patients diagnosed at least after 9 day of the onset of fever; (5) NS1 antigen alone may be positive indicating primary dengue fever patient diagnosed in very early stage *i.e.* during first four days of fever and (6) IgG reactive+NS1 positive indicating secondary dengue fever patients during first four days of fever.

In dengue patients beyond 9 d only IgM or IgG or both IgM and IgG antibodies will be found indicating primary dengue or old dengue cases or secondary dengue cases respectively.

So the sample timing and the need for using dengue specific antigen NS1 and both the antibodies (IgM and IgG) are very important criteria that will widen diagnostic horizon. When these tests are performed together the status of a dengue patient can be precisely diagnosed in a laboratory set up helping to treat and monitor the patient.

The important outcome was that the status of a dengue patient could be correctly evaluated in laboratory practice if all three tests were done simultaneously, for better monitoring and effective treatment of dengue cases which also, when employed in sero epidemiological survey would be able to detect all cases of dengue (such as early or late infection, primary and secondary infection). Another outcome was the fact that for diagnose of dengue after 5 d of fever, Mac-ELISA test was found to be better than NS1 antigen test.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Dengue has become a worldwide public health problem. Secondary infection in patients suffering results in severe form of the disease. The present study has been able to demonstrate this aspect from a recent dengue outbreak in Kolkata.

Research frontiers

The present study has clearly indicated the importance of the secondary infection in dengue infected patients.

Related reports

The data about the greater number of secondary infection of dengue have been indicated by Hati (2006) but could not be confirmed earlier. Present study has confirmed his findings.

Innovations & breakthroughs

This paper clearly indicated that secondary infection (almost two-third) in dengue will surely lead to more severe cases in the city.

Applications

This type of study has huge public health importance where proper measures can be taken to prevent such severity of the course of the disease.

Peer review

This is a good study in which the authors have clearly shown that secondary infection in dengue is highly prevalent in Kolkata city.

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