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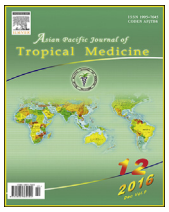

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NS1 antigen: A new beam of light in the early diagnosis of dengue infection

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ABSTRACT

Objective: To evaluate NS1 antigen detection ELISA for the early laboratory diagnosis of dengue virus infection.
Methods: The present study was conducted to evaluate the overall positivity of NS1 antigen detection ELISA and its comparison with viral RNA detection via real time PCR and IgM antibodies detection by ELISA.
Results: A total of 1270 serum samples were tested 86% (1097/1270) were detected positive by one or more than one diagnostic test. Out of 1 270, 64% (807/1270) were positive by NS1 ELISA and 52% (662/1270), 51% (646/1270) were positive by real-time RT-PCR and IgM ELISA respectively.
Conclusions: NS1 antigen detection ELISA is highly suitable diagnostic tools and it also has great value for use in outbreak and epidemic situation.

1. Introduction

Dengue fever is a mosquito borne arboviral infection caused by four serotypes of dengue virus (DENV) endemic in tropical and subtropical regions. Dengue virus contains single strand RNA genome that encodes three structural and seven non-structural proteins (NS). The non-structural protein NS1 is considered an important diagnostic marker for acute infection from the first day after onset of infection, whereas IgM is generally detectable after 4–6 d [1].

Currently a variety of diagnostic tests are available for the diagnosis of dengue infection, such as virus isolation, RT-PCR, real-time RT- PCR, viral antigen and antibodies detection by ELISA or rapid tests. In the present study we used the detection of soluble NS1 antigen by ELISA and compared its results with molecular and antibodies detection.

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2. Material and methods

From September 2013 to November 2015, a total of 1270 blood specimens were received from Khyber Pakhtunkhwa (KPK) Province and processed at the Department of Virology National Institute of Health Islamabad for the laboratory confirmation of dengue virus infection. After standard separation of serum, each sample was analyzed for the detection of NS1 antigen, detection (Platelia dengue NS1 ELISA) for viral RNA by serotype specific real-time RT-PCR [2] and for the detection of IgM antibodies (Panbio). Study was approved by the ethics Review Committee of National Institute of Health (NIH) Islamabad.

3. Results

During the study period (2013–2015), a total of 1270 serum samples were tested for NS1 antigen, viral RNA and dengue IgM antibodies. Out of 1270 serum samples tested, 86% (1097/1270) were positive by one or two diagnostic test. Of the 1270 total samples, 68% (866/1270) were from male and 32% (404/1270) from female; the male to female ratio was 2:1. Young adults in the age group of 16–30 years were most affected during 2013–2015 outbreaks. The seasonal trend of dengue cases was reflected by peak of positive cases detected during post monsoon season in 2013–2015. Concerning the results obtained with each

one of the three test used, 64% (807/1270) were positive for the NS1 antigen, 52% (662/1270) were positive for viral RNA by real-time PCR and 51% (646/1270) were positive for the IgM antibodies. Only 15% (173/1270) samples were tested negative when evaluated by all assays. From 1270 total samples, 37% (463/1270) were negative by NS1 ELISA. Of these 463 NS1 negative samples, 54% (248/463) were positive for IgM, on the other hand, 49% of the samples (624/1270) were negative for anti-dengue IgM; amongst them 66% (409/624) were positive for the NS1 antigen. Comparison of NS1 ELISA, IgM ELISA and Real-time RT-PCR is shown in Table 1. A total of 81% (615/761) of samples were detected positive on day 1–5 for NS1, 57% and 35% for real-time PCR and IgM respectively. Those collected on day 6–10, IgM detection increased up to 73% and NS1 antigen detection decreased to 42%. Detection of NS1 antigen also decreases down to 6% on the samples collected after days 10 of onset and IgM detection rate increased to 77% (Table 2). The sensitivities of NS1 antigen detection, viral RNA detection by real-time PCR and IgM antibodies detection related to number of days after onset of fever is shown in Figure 1.

Table 1

Comparison of NS1 ELISA, IgM ELISA, and real-time RT-PCR.

Parameter	*n*	IgM		Real time RT-PCR	
		Positive	Negative	Positive	Negative
NS1+	807	398	409	480	327
NS1−	463	248	215	182	281
Total	1270	646	624	662	608

Table 2

Percentage of positivity of different tests and its association with date of onset of illness [*n*(%)].

Days after onset of fever	Total samples tested	Positivity		
		NS1+	Real time PCR+	IgM+
1–5	761	615 (81)	432 (57)	264 (35)
6–10	445	188 (42)	212 (48)	333 (73)
>10	64	4 (6)	18 (28)	49 (77)

4. Discussion

The present study reports 3 outbreaks of dengue infection in the year 2013–2015 in KPK Province, Pakistan. The anti-dengue IgM capture ELISA is among the mostly used tests for the laboratory confirmation of infection in developing countries even if the overall sensitivity based on this test is quite low in early stages. Other diagnostic tests are less suitable due to costs and sophisticated laboratories and well trained staff requirement [3]. The overall 15% (173/1270) confirmed dengue negative cases in this study may be infected by other infections that clinically resemble dengue, such as malaria [4]. In the present study, a higher number of male were affected than female and highest number of cases were between 16 and 30 year of age observed correlated with the results of a study conducted by Halstead [5,6]. Furthermore, the maximum number of cases were reported during the month of September–November of the year 2013–2015 indicating that dengue infections are mostly reported in the post monsoon season, as expected and reported [7,8].

According to the results of present study, it was observed that many NS1 negative cases were found positive for IgM antibodies and some IgM negative cases were found positive for NS1 antigen; these results are in agreement with previously published data [9,10]. Therefore, these reciprocal results from both assays (NS1 and IgM ELISA) showed that the overall diagnostic capability to identify DENV infections can be increased by using a combination of these tests for sample collected after day 6–10 of illness particularly when only single blood sample is available and after 10 d IgM antibody ELISA is most suitable method for dengue diagnosis as reported previously [11,12]. A decreasing trend in the antigen detection corresponds with an increasing trend of antibody detection and there was a significant difference ($P < 0.005$) when the results of NS1 ELISA and IgM ELISA was compared with days of onset of illness as reported elsewhere [13,14].

In conclusion, the NS1 antigen detection gave overall higher sensitivity rate as compared to IgM ELISA and real-time PCR for the early laboratory confirmation of dengue infection.

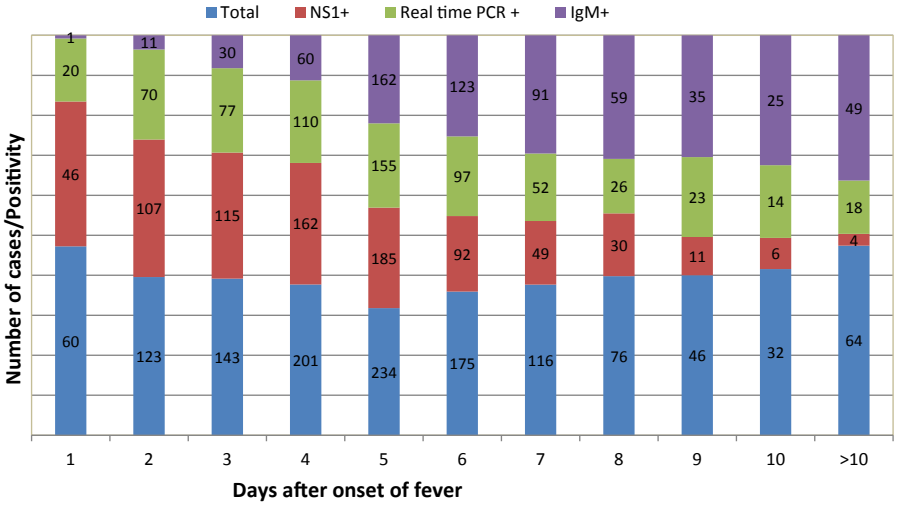

Figure 1. Positivity of NS1, real-time PCR and IgM in samples from suspected dengue cases and relationship of positivity with date of onset of infection.

Conflict of interest statement

We declare that we have no conflict of interest.

Author contributions

SSZZ, MS, RF conceived and designed the study. MS, SShaukat, SSharif, UBA, AK, MA analyzed data and wrote the manuscript. MS, MMA, YA, MU, MSufian, GM performed the laboratory tests. MS collected patients' information and performed analysis.

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