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RESEARCH ARTICLE

NS1: An early Diagnostic Tool for Dengue Virus Fever

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ABSTRACT

Dengue virus is a highly prevailing unremitting pathological menace in the developing countries like Pakistan. The variation in the type and frequency of dengue infection demands a continuous surveillance on its spread and diagnosis, in order to develop appropriate management and therapeutic strategies. Dengue Virus is highly complex disease with various manifestations. It is hard to characterize the dengue viral infection with ordinary laboratory tests. However, NS1 could serve a good diagnostic tool in the first few days of fever. In this research, 88 patients from Lahore regions of Punjab were analysed for the presence of dengue specific NS1 antigen from 1 to 30 days of dengue fever. ELISA based kit was employed. The obtained data was analysed expressed percentage frequency of NS1 positive and negative along with IgM positive and negative in all dengue fever patients. Importantly it was observed that 36.3 % were infected by dengue. NS1 antigen was efficiently quantified at earlier days of infection. IgM was negative in all the subjects who were positive for NS1 on the second day of fever. However, this association weakened with the progression of fever. It was analysed that thrombocytopenia and leuckocytopenia are not linked with the NS1 positivity and these states were random. It can be inferred that dengue diagnosis is very complicated and the antigenic profile dramatically changes with the progression of fever in terms of days. Clinicians should employ a diagnostic method based on a comprehensive analysis of subjects in order to minimize the risk of dengue shock syndrome resulting in haemorhage.

Keywords: Dengue virus fever, NS1, dengue outbreak in Pakistan, Viral borne diseases in Pakistan

Dengue is a widespread viral borne infectious fever in humans of the tropics and subtropics of the world (1). It has been estimated that near about 100 million cases, 500,000 cases of dengue haemorrhagic fever (DHF) and 12000 deaths occur worldwide annually due to of dengue fever (2). DENV has become the most important arthropod-borne virus affecting human (3).

In dengue infection, viremia usually hits the peak at the time or shortly after the onset of illness and can be detected within 2 to 12 days of illnes. The number of viral infected cells can determines the severity of illness and is directly related to antibody dependent enhancement (ADE) infection of peripheral leukocytes. It is also hypothesised that there is an association between dengue viremia illness and disease outcomes in patients with secondary dengue virus 1 and dengue virus 2 infections but there was no any association found in patients with primary dengue virus 1 infection. The severity of diseases is directly related to the dengue virus 3 viremia (measured as dengue virus 3 genome equivalent levels) and subsequent immune activation. There is also direct relationship between the magnitude of viremia and the magnitude of plasma leakage. Sensitive and reproducible quantitative RT-PCR

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assays have been reliably used to find out the severity of disease and viremia level (4, 5).

Flavivirus are single-stranded, positive-sense RNA enveloped viruses. Their genomic RNA is about 11 kb long and contains 10 genes encoding three structural proteins namely, capsid [C], envelope [E], and membrane [M]) and seven non-structural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5) (6). The polycistronic coding region is flanked by non-coding regions at its 59 and 39 ends. The non-structural protein, NS1, is a highly conserved glycoprotein, but its biological activity has not been established. During *in vitro* infection, the flavivirus NS1 protein is expressed as an intracellular membrane-associated form essential for viral replication (7) or as a cell surface-associated form that may be involved in signal transduction (8). The patients with DHF were found to have high levels of NS1 protein in their serum, therefore NS1 could be useful prognostic indicator in DHF.

In solution, secreted NS1 protein behaves as a hexamer; it circulates and accumulates in the sera of dengue virus-infected patients throughout the clinical phase of the disease (9, 10). A recent study demonstrated that soluble NS1 protein binds to endothelial cells and are recognized by anti-NS1 antibodies which could contribute to plasma leakage as the dengue virus infection severe more (11). The detection of secreted NS1 protein represents a new approach to detect acute dengue infection. A commercially developed diagnostic test based on dengue NS1 antigen-capture ELISA was investigated in two studies (one in South America with an overall sensitivity of 88.7% and the other one in Southeast Asia with an overall sensitivity of 93.4% and both of these test showed 100% specificity (12).The present research work is to evaluate the NS1 antigen in dengue infected patients and the level of viremia with dengue disease severity using well characterized sera from the patients presenting at hospitals.

MATERIAL AND METHODS

Sample Collection

During 2011 dengue epidemic in Lahore, Punjab, Pakistan, blood samples of 88 children who presented with fever and were suspected of being infected with dengue virus infection; were collected for the descriptive, prospective and retrospective questionnaire based study. Samples were collected from Dengue Ward, Children Hospital, Ferozpur Road, Lahore. Detailed physical examination was performed and all the available blood test reports were taken. Questionnaires were duly filled with bio data of the child, clinical presentation of the illness, Complete Blood Count (CBC) record, along with available additional investigative information. Children with diseases like diabetes, cirrhosis, CVD or kidney disease were not included in the study.

Biochemical Analysis

All the biochemical analysis were performed at the Centre for Research in Molecular Medicine (CRIMM), The University of Lahore. Serum was centrifuged at 4000 rpm for 10 minutes and then aliquoted and preserved at -20 °C. Serum concentration of NS1 antigen was measured by using commercial ELISA kits by BioRad (according to manufacturer protocol).

Statistical Analysis:

The significance of difference between quantitative variables was analysed by 2-tailed Student's t-test. Pearson test was used to calculate correlation between variables of interest. P value < 0.05 was considered statistically significant. All calculations were carried out with the SPSS version 19 (SPSS, Inc, Chicago, IL, USA).

Table 1:
Expression Pattern of NS1 and IgM in 80 Dengue Virus Fever Patients at Different Days (1 to 30)

Days of fever	Total	%age	NS1 +ve	%age of NS1+	IgM +ve	% of IgM+	NS1 -ve	%age	IgM -ve	%	Equivocal	%
1	5	5.6	1	20.00	1	20.00	4	80.00	4	80	-	-
2	7	7.9	5	71.43	0	0.00	2	28.57	7	100	-	-
3	15	17	7	46.67	8	53.33	8	53.33	7	46.6667	-	-
4	12	13.6	3	25.00	11	91.67	8	66.67	1	8.33333	1	8.33
5	14	16	4	28.57	12	85.71	10	71.43	2	14.2857	-	-
6	8	9	3	37.50	7	87.50	4	50.00	1	12.5	1	12.50
7	12	13.6	5	41.67	8	66.67	7	58.33	4	33.3333	-	-
8	3	3.4	1	33.33	1	33.33	1	33.33	2	66.6667	1	33.33
10	2	2.2	-	-	1	50.00	2	100.00	1	50	-	-
11	1	1.1	-	-	1	100.00	1	100.00	0	0	-	-
12	3	3.4	-	-	3	100.00	3	100.00	0	0	-	-
14	2	2.2	1	50.00	1	50.00	-	-	1	50	1	50.00
20	2	2.2	1	50.00	2	100.00	1	50.00	0	0	-	-
30	2	2.2	1	50.00	2	100.00	1	50.00	0	0	-	-

RESULTS

Total number of patients included in the study was 88. Frequency of male subjects was 47 making 53.5% of total whereas; female subjects were 45.5% of total with representative frequency of 41. The male to female ratio came out to be 1.17:1.

The subjects which were analyzed on the basis of NS1 antigen were descriptively analyzed based on their day of fever. It was observed that NS1 was detected in 71.43% of the total subjects on 2nd day of fever. However this efficiency decreased with increasing days and more number of samples were diagnosed negative by NS1 ELISA. It was also observed that on day 2 IgM and NS1 were 100% correlated.

DISCUSSION

Provision of proper treatments to the patient depends largely upon an early laboratory diagnosis of acute dengue virus infection. The patient may die of complications within first 24 hours if this integral component is overlooked. Currently various methods are available for the diagnosis of severity of the disease. However, there is a severe deficiency of complete data about the diagnosis and pathogenesis of the virus in association with period of illness. Moreover, little information is in hand about antigenic presentation of dengue fever in children of the Pakistani population.

In the present study, children suffering from fever were suspected of dengue virus infection. They were tested with dengue specific NS1 ELISA kits for diagnosis of dengue.

Figure 1A: Detection of NS1 in 80 Dengue Virus Patients From 1 to 30 days of Fever

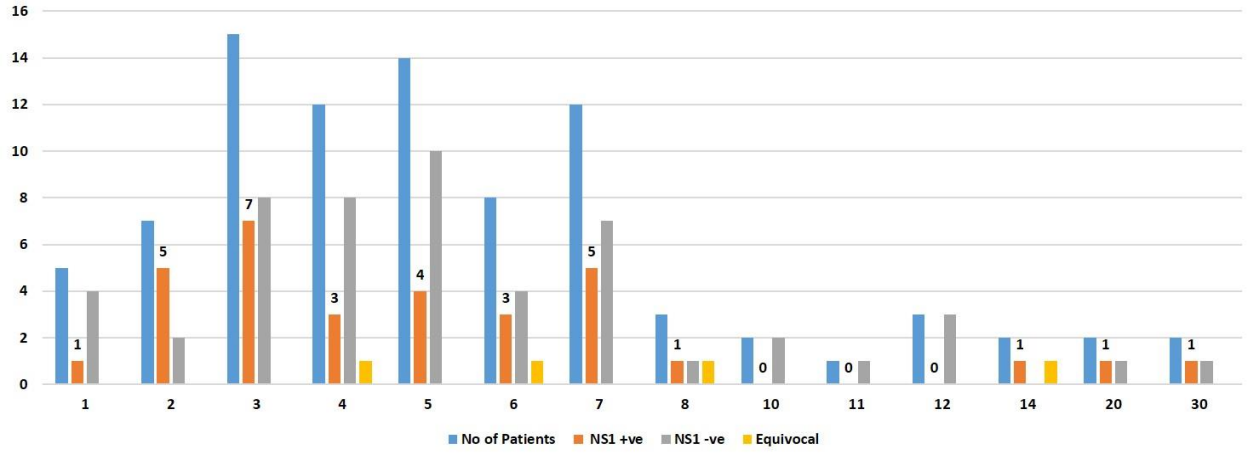
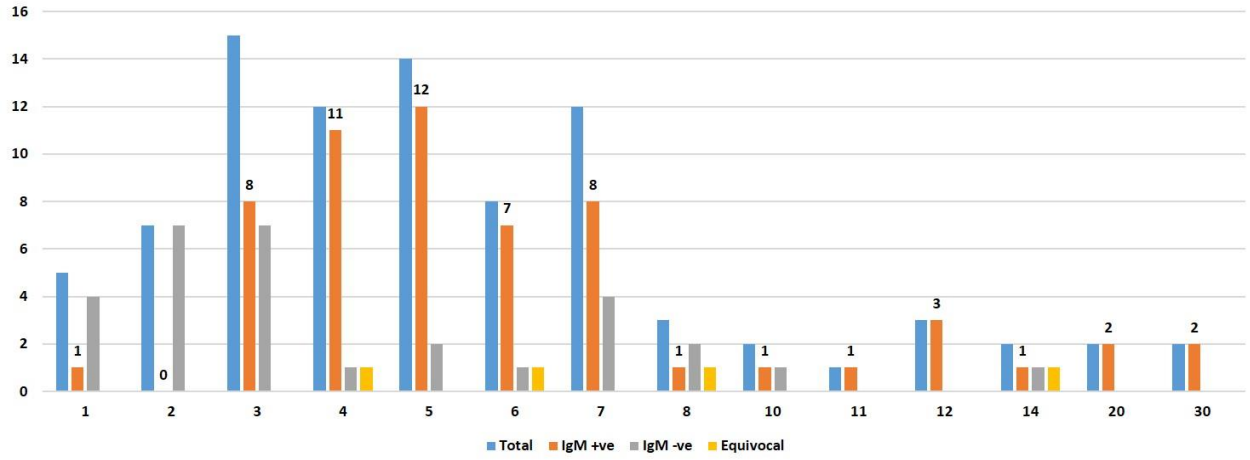


Figure 1B: Detection of IgM in 80 Dengue Virus Patients From 1 to 30 days of Fever



Previous studies have reported sensitivity greater than 90% for diagnosis of dengue fever. Based on the test, the subjects were then classified into dengue positive and dengue negative subjects.

Based on WHO's classification of three phases i.e. acute phase (2-7 days after onset of fever), afebrile phase (8-11 days after fever onset) and convalescent phase (18 days onwards), among 73 patients in the first phase, 28 were dengue positive with 76.71% of total and 43 individuals were dengue negative with 58.0% of total. All the subjects found positive and negative for NS1 antigen were reassessed with respect to the day of presentation of fever. It was observed that NS1 was clearly detected in early febrile phase and the ability decreased with increasing day of fever.

Also, IgM correlated well with NS1 in early days but later the association was poor.

CONCLUSION

Dengue virus is highly complex disease with various manifestations. It is hard to characterize the disease with various tests. However, NS1 could serves a good diagnostic tool in the first few days. It was also concluded from the current study that thrombocytopenia and leucocytopenia is because of the serotype and is not particularly associated with any antigen and antibody.

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