

## ROLE OF NS1 ANTIGEN IN DIAGNOSIS OF ACUTE DENGUE INFECTION

LATIF S.,<sup>1</sup> ANWAR M.S.,<sup>2</sup> JAFFERY G.<sup>3</sup> AND SALEH M.B.<sup>4</sup>

<sup>1,3,4</sup>Services Institute of Medical Sciences, <sup>2</sup>CMH Lahore Medical College, Lahore – Pakistan

### ABSTRACT

**Background and Objectives:** The study was designed to assess the sensitivity of NS1 antigen ELISA test in Dengue fever in patients whose samples were received in the Microbiology Section, Department of Pathology, SIMS / Services Hospital, Lahore from October to December 2011.

**Methodology:** Samples from clinically suspected cases of acute dengue fever irrespective of age and sex with available history of “day of fever” were included in this study. One hundred and thirteen blood samples were received in properly labeled disposable syringes along with request forms detailing patient information including the “day of fever”. Dengue NS1 antigen ELISA tests were performed on the sera on the same day.

**Results:** The NS1 antigen ELISA test positivity was 50 – 81 percent in the first six days of infection with dengue virus. After that, there was a gradual decline in the NS1 antigen positivity.

**Conclusions:** NS1 antigen ELISA test has a high sensitivity in the first week of dengue infection. Therefore, it is suggested that laboratories that do not have sophisticated equipment or lack funds to do PCR can perform NS1 antigen ELISA test.

**Key Words:** Dengue Fever; Dengue haemorrhagic fever; Dengue NS1 antigen; “day of fever”.

### INTRODUCTION

Dengue fever (DF) is caused by flavivirus, which comprises of four serotype: DEN-1, DEN-2, DEN-3 and DEN-4. It is transmitted by the Aedes mosquito. Since 1990, DF has become the most important mosquito borne disease in the world, second only to malaria.<sup>1</sup>

Pakistan first reported an epidemic of dengue fever in 1994 in Karachi. Since then, it has become endemic in our country. While the serotype DEN-2 is predominantly seen, the co-circulation of DEN-2 and DEN-3 serotypes was responsible for the 2006 outbreak in Karachi.<sup>2</sup> Mini-epidemics of dengue fever occurred in Lahore in 2007, 2008 and 2009; they were caused by the DEN-2 and DEN-3 serotypes of the virus.<sup>3</sup> These outbreaks led to many people becoming susceptible to the more severe form of DF i.e. dengue haemorrhagic fever (DHF) as they had preexisting non-neutralizing heterologous dengue antibodies.<sup>4,5</sup>

According to WHO guidelines for diagnosis, treatment, prevention and control of dengue 2009,<sup>6</sup> confirmation of this disease requires different tests depending on the stage of the disease. In the first few days of infection, diagnosis is based on viral culture and molecular methods such as reverse transcriptase polymerase chain reaction. These techniques, while rapid and effective in providing early dengue diagnosis, are costly and require trained personnel and the facilities to perform.

After 5 days of fever, indirect serodiagnosis, based on seroconversion of IgM or a four – fold rise in IgG, is possible. However, in secondary infections, diagnosis may sometimes be missed because IgM levels are low or undetectable. Moreover, the Ig M could be from a previous infection and may remain positive for three months.<sup>7,8</sup> For IgG, two samples are required from both the acute and convalescent stage. This results in a delay in confirming the diagnosis. The more recent development of DENV non-structural protein 1 (NS1) antigen detection by enzyme immuno-assay has offered clinical laboratories an effective tool for diagnosis of acute DF.<sup>9</sup> This is important as most of the hospital laboratories in Pakistan have the facility for ELISA tests but not for PCR or viral cultures.

In this paper, analysis of the results of the NS1 antigen ELISA test carried out for the diagnosis of DF/DHF was done. The samples were obtained from patients admitted in Services Hospital whose clinical features were suggestive of the disease as defined in the WHO guidelines.<sup>6</sup>

### MATERIALS AND METHODS

Samples were received from 113 patients, 64 males and 49 females. Eighty – four percent of the patients were more than 18 years old. They had presented to the Emergency Department with DF / DHF and were admitted in different wards at Services Hospital Lahore,

from 20<sup>th</sup> October to 30<sup>th</sup> November 2011. The Microbiology Laboratory SIMS received blood samples in a disposable syringe. The blood samples were allowed to clot before being centrifuged at 4000 RPM to separate the sera. NS1 antigen ELISA tests were performed. For this, ELISA kits manufactured by BIORAD France were used; the tests were performed following the instructions given by the manufacturers. The remaining sera were stored in properly labeled serum cups at -20°C.

## RESULTS

**Table 1:** The sensitivity of NS1 antigen according to the day of fever.

Day of Fever	No. of Cases	NS1 Antigen +ve	Percentage
1	6	3	50%
2	16	13	81%
3	3	2	66%
4	12	6	50%
5	27	15	55%
6	22	11	50%
7	12	5	40%
> 8	15	5	33%

In the first six days of infection with dengue virus, 58.1% of the patients tested positive for NS1 antigen. Maximum positivity was seen on day two of infection (81%). After that, there was a gradual decline in the NS1 antigen positivity. There was however, no statistically significant difference between positivity in first five / six days as compared to later days of infection.

## DISCUSSION

Early diagnosis and thus anticipation of the critical phase of DF / DHF may help clinicians properly manage and reduce morbidity and mortality in such patients.<sup>9,10</sup> In this study NS1 antigen tests performed on clinically suspected cases of dengue fever were analysed. We found NS1 antigen detection to be very helpful as it was positive in 50-81% patients during the first six days of fever. The variation in the test positivity on different days may be due to poor reporting of the day of fever or infection with a different serotype. The sensitivity for NS1 antigen of different viral serotypes varies.<sup>11</sup> The variation may be due to the fact that the antibodies raised and purified are against one particular serotype NS1. These may react less efficiently against other serotypes because the homology in the amino acid sequence of NS1 does not exceed 80% among dengue viruses.<sup>9,12</sup> In the present analysis of results the highest sensitivity was seen on day 2 of fever, that is 81%

(13/16 patients).

This finding is comparable to a study conducted in Thailand on patients infected with DEN-2 serotype of the virus: out of the 32 children, 28 (88%) had detectable free sNS1 during the febrile phase of illness. Among the patients with three days of fever, 10 (71%) out of a total of 14 with DF and 16 (89%) out of the 18 with DHF had detectable free sNS1 levels.<sup>13</sup>

In a study carried out to assess different diagnostic tests, 237 single acute serum specimens and 50 paired specimens were tested. The sensitivity of NS1 antigen ELISA test was found to be 70.6%.<sup>14</sup> In an evaluation study for NS1 antigen enzyme immunoassay, 239 serum samples were taken from patients with acute infections who had tested positive for one of the four – dengue virus serotypes by reverse transcription – PCR and / or virus isolation. The sensitivity of the NS1 antigen was 88.7% and none of the serum samples from patients not infected with dengue virus tested positive.<sup>12</sup>

Other workers have found a higher positivity rate, as in the study conducted by Chuansumrit A.<sup>15</sup> in 2008. They found NS1 antigen positivity at 100% (7 out of 7) on day 2, in the sera of patients with either DF or DHF. As the sample size is small, the results may not be truly representative of the situation.

The sensitivity of the tests remains variable on different days of fever in most of the studies. However, the general trend is that it decreases after day 6. This is either due to the clearance of the virus by the neutralizing antibodies or due to the formation of immune complexes.<sup>12</sup> Likewise, in the current analysis of the data, our results show a decline in positivity for NS1 antigen after day 6.

Although the true role of NS1 in viruses or the pathophysiology of DF / DHF in humans is not known, it has been suggested that the presence of higher levels of NS1 is an indicator of DHF. Therefore, in the future, effort should be made to quantitatively analyze the NS1 antigen and correlate with the clinical picture.<sup>9,12,14</sup>

It is **concluded** that NS1 antigen ELISA test is a sensitive confirmatory test available for diagnosis of acute dengue fever. It is suggested that the laboratories that do not have sophisticated equipment or funds to carry out PCR test for dengue fever can use easy to perform and cheaper NS1 antigen ELISA test in first week of dengue fever.

## ACKNOWLEDGEMENTS

The authors are thankful to The Services Institute of Medical Sciences / Services Hospital, Lahore Ethical Committee for review and granting permission to publish this article.

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