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Dengue NS1 Antigen for Early Detection of Dengue Infection

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A B S T R A C T

Dengue is a mosquito-borne viral disease affecting tropical and subtropical regions of the world. Symptoms may range from a mild undifferentiated febrile illness to dengue fever (DF) and dengue haemorrhagic fever (DHF), a potentially life threatening disease. Detection of non-structural antigen (NS1 Ag) may help in the early diagnosis and treatment of dengue. Aim of the study is to detect dengue infection by NS1 Ag detection and also to know its prevalence. A retrospective study was carried out in a tertiary-care hospital in Chamarajanagar, Karnataka, India. The serum samples from 787 suspected dengue cases which included patients of all age group and from both gender, were subjected to dengue NS1 antigen detection by microwell enzyme-linked immunosorbent assay (ELISA). Of 787 serum samples tested, 286 (36.34%) samples were positive for NS1 antigen. Maximum number of positive cases were in age groups of 0-18 years 70 (24.48%) followed by 19-45 years 69 (24.12%) with no significant difference between males 64 (54.70%) and females 53(45.30%). Prevalence of dengue 36.34% is high in our locality. So a prevention strategy has to be taken. NS1 Ag detection has a significant role in early diagnosis of dengue fever. Hence, it is recommended to do this test for early diagnosis and treatment.

Introduction

Dengue virus infection has emerged as a notable public health problem in recent decades in term of the mortality and morbidity associated with it (Neralwar *et al.*, 2015). It is the most common arboviral illness in humans (Amol Hartalkar *et al.*, 2015). Dengue (DEN) virus belongs to the genus Flaviviruses, and consists of four serotypes: serotype 1 (DEN-1), serotype 2 (DEN-2), serotype 3 (DEN-3), and serotype 4 (DEN-4) (Aditi Garg *et al.*, 2015). Infection to one serotype confers immunity only to that particular infecting serotype. Subsequent infection with one of the three remaining serotypes results in immune-enhanced disease in the form of severe hemorrhagic fever or dengue shock syndrome (Purimitla Usha Rani *et al.*, 2017). Worldwide, an estimated 2.5 billion people are at risk of infection. It is estimated that more than 50 million infections occur each year, of which 500,000 hospitalizations are of dengue haemorrhagic fever, mainly among children, with the case fatality rate exceeding 5% in some areas (Prakash Babaliche *et al.*, 2015). Dengue is known in India since 1940s, but the disease is very limited in its spread. Dengue is becoming rampant in many states of southern

India. As of now, no specific treatments or vaccines are available against the disease (R. Chandran *et al.,* 2015). Dengue viruses are transmitted to humans by *Aedes aegypti* mosquitoes and cause a wide range of symptoms, from unapparent or mild disease (dengue fever) to a severe hemorrhagic form (dengue hemorrhagic fever and dengue shock syndrome) (Philippe Dussart *et al.,* 2006).

Diagnosis of acute dengue infection using clinical signs and symptoms is complicated by the wide range of possibilities for differential diagnosis, and therefore, laboratory assays are normally relied upon to make a diagnosis (Stuart D. Blacksell *et al.,* 2012). Current diagnostic methods are often unable to recognize emerging epidemics in a timely manner or at a reasonable cost, drastically reducing the efficacy of control measures (Kovi Bessoff *et al.,* 2008).

Detection of the dengue virus by virus isolation or by nucleic acid detection methods are considered as confirmatory tests for confirming the diagnosis of dengue infection. However, due to the need for advanced laboratory facilities these two methods may not be suitable for routine diagnosis of dengue virus infection early in the disease in resource poor communities (Paranavitane *et al.,* 2014). Serological diagnosis offers many advantages including more flexible schedules for testing, lower cost, and more widely available reagents. But, cross-reactivity between other flaviviruses and "original antigenic sin" complicate specific diagnosis of secondary flavivirus infections (Kovi Bessoff *et al.,* 2008).

Non-structural 1 (NS1) protein, which is approximately 45 kDa in molecular weight, is secreted from dengue virus infected cells. Dengue NS1 antigen is an important diagnostic biomarker found circulating in patient blood samples (Wei Ru Wong *et al.,* 2016). The NS1 antigen is found together with endothelium, free or soluble in the sera of patients, from one day before the onset of symptoms and can be detected at least up to five days after the onset of symptoms, allowing for an early diagnosis (Bisordi *et al.,* 2011). An enzyme-linked immunosorbent assay, specific to dengue virus type 1 nonstructural protein NS1, has been developed for detection of dengue NS1 antigen during the acute phase of disease in patients experiencing primary and secondary infections (Kumarasamy *et al.,* 2007). In the absence of a vaccine, dengue prevention is focused upon controlling mosquito vectors (Natalia V. Voge *et al.,* 2013). Controlling dengue infections is challenging because it requires not only effective control of vectors responsible for transmitting the virus but also accurate and rapid diagnosis (Fauziah Md Kassim *et al.,* 2011).

Materials and Methods

A retrospective study was conducted at Chamarajanagar Institute of Medical Sciences, Chamarajanagar. The age, gender and results of dengue NS1 Ag ELISA test were collected from the laboratory registers. The data were entered into Excel for analysis. Permission was obtained from the institution. As the study was based on secondary data, there were no ethical issues.

With aseptic precautions, blood samples were collected from clinically suspected dengue cases. The serum was separated by centrifugation of the whole blood sample and if delay in testing, stored in the refrigerator at -20^{o}C. NS1 antigen is detected by using Qualisa, a microwell enzyme immunoassay for dengue NS1 from Qualpro diagnostics (A division of Tulip Diagnostics (P) Ltd., phase II C, Verna Industrial Estate, Verna, Goa, India). The tests were carried out following the manufacturer instruction.

Results and Discussion

A total of 787 dengue suspected serum samples were tested, of which 286 (36.34%) samples were positive. Maximum number of cases were from age groups 0-18 years 148 (51.75%) and 19-45 years 121 (42.31%) followed by > 45 years group 17(05.94%), which is very less. There was no significant difference between males 140 (48.95%) and females 146 (51.05%) (Tables 1 and 2).

Dengue virus infection most commonly affects tropical and subtropical regions of the world. Epidemics of dengue infection are showing an increasing trend in recent years (Ashwini Manoor Anand *et al.,* 2016). With the escalating incidence of dengue infections and the absence of vaccines for the prevention of this disease, early diagnostic confirmation of dengue virus infections in patients is needed, as it allows for timely clinical intervention, etiologic investigations, and disease control. Hence, diagnosis of dengue disease during the acute phase should be a priority for patients and for public health reasons (Seok Mui Wang *et al.,* 2010).

The study done by Paranavitane *et al.,* (2014), found that platelet counts were lower in those who were NS1 antigen positive at the time of admission, but this was not significant. However, interestingly serum NS1 antigen levels significantly correlated with a serum interleukin (IL)-10 levels which are suggested as a possible marker of severe clinical disease. Currently, most laboratory dengue diagnosis is achieved by DENV isolation, RNA detection, ELISA for anti-DENV IgM and IgG antibodies and non-structural protein 1 (NS1) (Sundaram *et al.,* 2016).

Apart from these traditional tests, latest advances such as use of biosensors are helpful in diagnosis (Pawar R *et al.,* 2015). Inspite of availability of current diagnostic tools, there is a need for reliable and dependable tools that are relatively easy to use and that do not require highly skilled personnel or costly equipment (Meng Ling Moi *et al.,* 2013).

Table.1 Prevalence of dengue cases

Total no. of samples tested	No. of positive samples NO. (%)
787	286(36.34)

Table.2 Age and gender wise distribution of dengue cases

Age (years)	No. of positive samples NO. (%)	Gender	
		Males NO. (%)	Females NO. (%)
0 - 18	148(51.75)	78(27.27)	70(24.48)
19 - 45	121(42.31)	52(18.18)	69(24.12)
> 45	17(05.94)	10(03.50)	07(02.45)
Total	**286(100)**	**140(48.95)**	**146(51.05)**

Kulkarni RD *et al.,* reported 40.6% positivity for any one of the dengue markers (IgM, IgG and NS1 antigen) by immune chromatography (Poongodi Lakshmi *et al.,* 2014). In present study, dengue was detected in 36.34% which is significant. Out of 286 seropositive patients, 140 (48.95%) were males and 146 (51.05%) were females. The most affected age group was 0-18 years 148 (51.75%) and 19-45 years 121 (42.31%) followed by > 45

years group 17(05.94%). NS1 Ag circulates uniformly in all serotypes of dengue virus and at high level during the first few days of illness (Syed Irfan Ahmed *et al.*, 2010).

Its levels range from 0.004 to 2µg/ml in acute phase serum, to only 0.004µg/ml or less in convalescent-phase serum samples (Ashwini Manoor Anand *et al.*, 2016). Along with the virus and viral RNA, it is detectable before the appearance of IgM and IgG antibodies in first infections and also before IgM in subsequent infections (Jacqueline Gosink. 2014). Enzyme-linked immunosorbent assay (ELISA) was developed to detect this viral protein (NS1 Ag). This assay has become a sensitive, specific test and is relatively inexpensive as compared to molecular diagnostics assays (Shamala, 2015). But here it is important to understand that, NS1 antigen detection assay has an advantage of detecting infection very early, however it disappears early also and is of little use in the early convalescence phase when IgM is useful (Pramod S. Manthalkar *et al.*, 2017). Also, these serological tests are unable to distinguish the serotype of dengue virus causing the infection. Polymerase chain reaction (PCR) is fast becoming the method of choice for the rapid detection of dengue viruses, especially in reference and research laboratories. With the recent advances in real-time PCR, several methods have been developed using this versatile tool that enables rapid detection, serotype identification, as well as viral RNA quantitation (Kwoon-Yong Pok *et al.*, 2010).

Dengue was detected in 36.34% which is significant and hence necessary efforts have to be taken for its prevention. NS1 Ag detection by microwell ELISA, which has high sensitivity and specificity, is very helpful in early diagnosis of dengue fever. It is recommended to do this test in all suspected dengue cases for early diagnosis and initiation of necessary treatment.

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