Original Research Article

Seroprevalence and evaluation of IgM and NS1 Ag detection for early diagnosis of dengue cases in a tertiary care hospital.

Abstract:

Introduction: Dengue fever, a mosquito borne illness, have grown to pose a serious danger to public health systems in developing nations. The Dengue virus (DENV), a member of the Flavivirus genus has four serotypes that cause the disease which range in severity from subclinical infections to severe disease with high morbidity & mortality. This study aims to assess the seroprevalence of dengue fever cases in a tertiary care hospital from East- Delhi region.

Material & Methods: This study was a retrospective observational study conducted in the Department of Microbiology at UCMS & GTBH. The data was collected from laboratory records from a period of Jan 2019 to December 2019.

Results: A total of 1449 clinically suspected cases of dengue were tested for the presence of NS1 antigen and IgM antibodies by capture ELISA. This study showed 32.22% (467/1449) seropositivity of dengue infection. Out of 1272, a total of 456 samples were positive for NS1 Ag ELISA. Whereas, out of 177, a total 11 samples were positive for IgM ELISA.

Observation: The study highlights the importance of early diagnosis and prompt management of dengue cases to prevent severe disease and reduce morbidity and mortality. This underscores the need for increased awareness, early detection, and appropriate management of dengue fever in endemic areas. especially during the peak months of October and November.

Keywords: *Aedes* mosquito, Dengue virus, Enteric Fever, Seroprevalence, IgM

Introduction:

Dengue fever, a mosquito borne illness, have grown to pose a serious danger to public health systems in developing nations. The vector borne viral disease is primarily transmitted by two most common *Aedes* mosquito (*Aedes aegypti* and *Aedes albopictus*) [1]. The Dengue virus (DENV), a member of the Flavivirus genus has four serotypes that cause the disease which range in severity from subclinical infections to severe disease with high morbidity & mortality. Despite the fact that the most of dengue fever episodes are mild and self-limiting, it can nevertheless result in deadly complications such as DHF (Dengue Hemorrhagic Fever) and DSS (Dengue Shock Syndrome) [2]. The asymptomatic cases in the earlier stages of

infection play a major role in the maintaining transmission of the disease in the community resulting in outbreak [3] Typical symptoms of dengue include a high-grade fever, headache, discomfort in the muscles and joints, aches, retroorbital pain, and skin rashes [3]. Severe dengue symptoms result from plasma leakage, hemo-concentration leading to hemorrhagic shock, and multiple organ failure with high fatality. The National Centre for Vector Borne Diseases Control (NVBDCP) has provided case definition for the disease based on symptoms & laboratory finding and is a notifiable disease under Integrated Disease Surveillance Project (IDSP) disease survey [4].

Serology, Virus isolation and molecular methods like Nucleic Acid Amplification Test (NAAT) are some of the diagnostic modalities currently available to detect dengue infection. NAATs and viral culture are expensive & specialized infrastructure is needed therefore have limited diagnostic use. For early diagnosis and intervention in resource limited settings, cost effective tests like ELISA are routinely used to identify dengue infection [4].

As per NVBDCP, India reported a total case 157315, 44585 and 193245 during the year of 2019, 2020 and 2021, respectively [5]. The total death reported during the period was 166, 56 and 346, respectively [5]. Delhi reported a total case 5077, 1269 and 13089 during the year of 2019, 2020 and 2021, respectively and only 23 deaths in 2021 [5]. Hence, this study was aims to assess the seroprevalence of dengue in the patients treated at a tertiary care hospital from East- Delhi region during 2019.

Material & Methods:

This study was a retrospective observational study conducted in the Department of Microbiology at UCMS & GTBH. The demographic and lab data were collected from medical records from Jan 2019 to December 2019 of patients who were clinically suspected of dengue.

Blood samples (3ml) taken from patients with clinical suspicion of dengue was submitted to the microbiology department. Blood samples were stored in the refrigerator at 4°C -8°C and processed for serum separation within 24h. Serum was separated by centrifuging blood at 3,000 rpm for 5min. serology test were performed for NS1Ag and IgM antibodies by using dengue NS1Ag MICROLISA (IR031096) and MACELISA kit (Panbio Dengue IgM Capture ELISA). The positive control and negative control from the test kit were put up. A Bio-Rad ELISA reader was used to read the ELISA microtiter plates. Optical density measurements were taken examined, and the result were interpreted in accordance with the manufacturer's recommendations.

Results:

A total of 1449 clinically suspected dengue cases were tested for the presence of NS1 antigen and IgM antibodies by capture ELISA. This study showed 32.22% (467/1449) seropositivity of dengue infection. Out of 1272, (total tested for NS1Ag ELISA)456 samples were positive. Whereas, out of 177(total tested for IgM ELISA)11 samples were positive.

Table 1 showed that from the total positive cases, Males (51.82%, 242/467) were more affected than females (48.17%, 225/467). Most common affected age group was 21-40 years (43.89%, 205/467), followed by 6-15 years (25.26%, 118/467) of age group (Table 2). Majority of positive dengue patients were from IPD (93.79%, 438/467) than OPD (6.20%, 29/467) (Table 3). Table 4 depicts the monthly distribution of positive cases. Maximum cases were recorded in October (30.40%, 142/467) followed by November (27.19%, 127/467).

Table 1. Gender-wise distribution of dengue cases

GENDER	NS 1 Ag ELISA		IgM Ab ELISA	
	Total number	**Positive**	**Total number**	**Positive**
Male	499	236 (51.75%)	62	6 (54.55%)
Female	773	220 (48.25%)	115	5 (45.45%)
Total	**1272**	**456**	**177**	**11**

Table 2. Age-wise distribution of dengue cases

AGE	NS 1 Ag ELISA		IgM Ab ELISA	
	Total Number	**Positive**	**Total Number**	**Positive**
0-5	53	17 (3.75%)	12	01 (9.09%)
6-15	236	115 (25.21%)	23	03 (27.27%)
16-20	169	66 (14.47%)	24	01 (9.09%)
21-40	658	201 (44.07%)	93	04 (36.36%)
>41	156	57 (12.5%)	21	02 (18.19%)
Total	**1272**	**456**	**177**	**11**

Table 3. Incidence of dengue cases from the IPD& OPD wards

Department	NS 1 Ag ELISA		IgM Ab ELISA	
	Total number	**Positive**	**Total number**	**Positive**
IPD	1175	428	170	10
OPD	97	28	07	01
Total	**1272**	**456**	**177**	**11**

Table 4. Month-wise distribution of dengue infection

MONTHS	IgM Ab ELISA	NS1 Ag ELISA

	Total Samples	**Positive Rate**	**Total Samples**	**Positive Rate**
Jan	19	06 (31.57%)	132	23 (1.74%)
Feb	00	00	34	02 (5.88%)
Mar	17	00	01	00
Apr	36	00	00	00
May	40	00	00	00
Jun	11	00	44	01 (2.27%)
July	00	00	105	25 (23.8%)
Aug	00	00	140	26 (18.57%)
Sep	00	00	251	105 (41.83%)
Oct	00	00	293	142 (48.46%)
Nov	00	00	253	127 (50.19%)
Dec	54	05 (9.25%)	19	05 (26.31%)
Total	**177**	**11 (6.21%)**	**1272**	**456 (35.84%)**

DISCUSSION:

Dengue NS1 antigen, a highly conserved glycoprotein which is produced in both membrane-associated and secretion forms, is abundant in the serum of patients during the early stages of DENV infection [4]. It's been discovered to be helpful in recognizing the presence of acute dengue infections. It is a simple test that is specific and shows high sensitivity. Early case detection with NS1, or when the infection is in the viremic stage is important form an epidemiological perspective to prevent transmission of the infection. For DENV, the NS1 ELISA-based antigen assay is commercially available, and the sensitivity and specificity of this assay has been assayed previously. Moreover, the specificity of NS1 assay helps in

differential diagnosis of various flaviviruses [4]. Considering the cost effectiveness of this test and ease of performance as compared to molecular diagnostic test, the Mac ELISA gives a practical advantage in resource limited settings.

IgM-Capture (MAC-ELISA) detect the anti-dengue IgM antibody develops within 5 days of fever though it varies between patients. Detectable IgM may persist in some primary infections for up to 90 days, but in the majority of patients, it declines to undetectable levels by 60 days [4]. Nevertheless, it verifies the presence of infection in the person at some point in the recent past months. MAC_ELISA is now widely used as a potential tool for DF/DHF surveillance. Hospitalized patients, who are frequently admitted at the end of their illness and have detectable IgM levels in their blood, can benefit the most from it [4]

The study shows a high seropositivity rate (32.22%) for dengue infection among clinically suspected cases during 2019. This result was higher compared to the study done by Kumar e al, which shows the seropositivity of dengue infection in 23% cases [6]. This highlights the higher burden and need for accurate and timely diagnosis of dengue fever, especially in endemic areas.

In the present study, the male to female ratio of dengue cases was 1.07:1 (242/225) with a male (51.82%) predominance than female (48.17%). However, the difference between the two groups was not statistically significant and this result is similar to the Indian studies which also showed no difference in the gender distribution of dengue cases [7,8].

The most commonly affected age group was 21-40 years, followed by 6-15 years. Indirectly the need for targeted interventions to prevent and manage dengue fever in these high-risk groups. A study done by Badoni G *et al*, found that the most positive cases for DENV are in the 21–30-year age group [9].

The predominance of IPD dengue cases (93.79%) compared to OPD cases (6.20%) raises concerns about the severity of the disease. It suggests that a significant number of patients require specialized care and monitoring. Similarly, a study done by Singh T et al, also reported that out of 108 positive cases, most of the cases from the IPD ward (66.66%, 72/108) and rest of the positive cases from OPD 33.33%,36/108) [10]. The difference in the burden of dengue cases in IPD vs OPD emphasizes the need for an early detection and prompt treatment, in order to reduce its impact on healthcare facilities.

This study also noted that the majority of positive cases occurred during the months of October and November. A seasonal variation as reported from other endemic areas, where transmission peaks during the rainy season. Post-monsoon months are more favorable for the vector to breed due to the stagnation of rainwater. The findings underscore the importance of targeted surveillance and control measures during the peak transmission season. This result was also represented by many other Indian studies done by Badoni G *et al*, Kalpana S *et al* and Badoni G, Gupta P *et al* [9,11,12].

The NVBDCP data reveals the fluctuating nature of dengue cases in Delhi, with a high number of cases in 2019, a decrease in 2020, and a significant increase in2021 [5]. Total 23

deaths were reported in2021 as per government data [5], which is lesser than the previous year's and thus it highlights the need for continued efforts to combat dengue, including mosquito control measures, public awareness campaigns, and accessible healthcare services to further reduce the impact of this mosquito-borne disease on the population.

The death rate associated with dengue in Delhi, India can vary from year to year and depends on various factor such as the severity of the outbreak, access to healthcare, and the effectiveness of preventive measures [5]. It is crucial for the government and healthcare authorities to continuously monitor and respond to outbreaks to minimize the impact on public health.

Conclusion:

In conclusion, the study demonstrated a high seropositivity rate for dengue infection among clinically suspected cases, and NS1 antigen ELISA test was more effective than IgM ELISA test for early diagnosis. The findings also suggest that males and younger age groups are more prone to dengue infection. Additionally, the study highlights the importance of early diagnosis and prompt management of dengue cases to prevent severe disease and reduce morbidity and mortality. This implies the need for increased awareness, early detection, and appropriate management of dengue fever in endemic areas. especially during the peak months of October and November.

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