

Significance of Serological Markers in Diagnosis of Acute Dengue Infection: 2021 Outbreak

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ABSTRACT

Objective: To highlight the significance of Dengue anti-IgM and Dengue NS1 antigen in diagnosing Acute Dengue infection.

Study Design: Cross-sectional study.

Place and Duration of Study: Virology Department, Armed Forces Institute of Pathology, Rawalpindi Pakistan, from Sep to Nov 2021.

Methodology: One hundred and fifty Dengue NS1-positive patients of either gender with age ≥ 5 years were included. ELISA tested patients for detection of Dengue anti-IgM antibody. A questionnaire was used to collect demographic and personal data of each patient.

Results: Out of 150 dengue NS1 positive samples, 26(17%) were positive for dengue anti-IgM. In collected samples, Dengue serological markers (NS1 and anti-IgM) positivity was observed based on 'the day of onset of symptoms. On days 1 and 2, 54 and 44 NS1 positive samples were collected, respectively, but no one was found positive for anti-IgM. Anti-IgM was detected on day-3 and onwards. 27% Dengue anti-IgM positivity was found on day-3, which increased gradually as the time of infection increased. On day four, it was 53%; on day 5, it was 55%; on day-6, it was 60%; on day 7, it was 75%; and on day 8, it was 100%.

Conclusions: The findings of present study suggested that anti-Dengue IgM antibodies are less significant than the Dengue NS1 antigen in diagnosing acute dengue infection.

Keywords: Acute dengue infection, Dengue NS1, Dengue anti-IgM, Serological markers.

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INTRODUCTION

Dengue is a significant human viral pathogen transmitted by mosquitoes, causing infection in tropical and subtropical countries worldwide. Approximately 50 million infections per year occur across 100 countries worldwide, with potential for further spread.^{1,2} In Pakistan, the first confirmed outbreak of Dengue Hemorrhagic Fever (DHF) was reported in 1994 by the Aga Khan University Hospital (AKUH); the reported serotype was DENV-2.³

The incubation period ranges from 3-14 days, and symptoms usually develop between 4th and seventh day. Fever is characteristic of dengue infection and lasts five to seven days.⁴ Symptoms may vary from nonspecific symptoms like rash and nose bleeding to more severe syndromes such as DHF and dengue shock syndrome (DSS).⁵ DHF is characterized by capillary leakage marked thrombocytopenia and bleeding diathesis, which may result in life-threatening hypovolemic shock (DSS).⁶

The laboratory diagnosis of dengue infection is based on detecting glycoprotein Dengue NS1 antigen,

dengue anti-IgM, anti-IgG and PCR to detect DENV RNA. The antibody production against DENV infection depends on the host's immune status.^{7,8} The NS1 is present at high concentrations in sera of dengue-infected patients during the early phase of the disease and remains positive from day 1-9 after the onset of symptoms. The IgM may become detectable on days 4-5 of illness in case of primary dengue infection and persists for 12 weeks, whereas IgG appears by the 14th day and may persist for life. Secondary infection shows that IgG rises within 1-2 days after onset of symptoms, simultaneously with IgM antibodies.^{9,10}

This study aims to determine the correlation and significance of Dengue anti-IgM with Dengue NS1 in Dengue viral infections during the 2021 outbreak. It is focused on the importance of diagnosing Dengue anti-IgM along with NS1 antigens, as it helps identify the stage of infection and the sensitivity and specificity of the results.

METHODOLOGY

After obtaining approval from the Institutional Review Board (BS AHS/VIR-4/READ-IRB/21/924), a cross-sectional study was conducted at the Department of Virology, Armed Forced Institute of Pathology,

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Rawalpindi Pakistan, from September to November 2021 during Acute Dengue infection outbreak in 2021. The sample size was calculated using formula, with the population proportion of Dengue virus = 11%.¹¹ The sampling technique was non-probability consecutive sampling.

Inclusion Criteria: Dengue NS1-positive patients of either gender with age ≥ 5 years were included.

Exclusion Criteria: Immune-compromised patients and patients having past Dengue infection as having a history of symptoms for more than two weeks.

A questionnaire was used to collect demographic and personal data of each subject. Confidentiality of the patient was maintained. The patient was prepared for the collection of samples with suspected dengue infection. Quality controls were considered, including positive and negative controls and quality assessment. Three millilitres of the patient's blood sample were withdrawn in a gel tube for dengue NS1 antigen and anti-IgM. The serum was separated by centrifugation at 3500 RPM for 4 minutes and then tested for serological markers (Dengue NS1 and Dengue anti-IgM).

Data was analyzed using the Statistical Package of Social Sciences (SPSS) v 23.0. Statistical Package for Social Sciences (SPSS) version 23.0 was used for the data analysis. Quantitative variables were expressed as Mean \pm SD and qualitative variables were expressed as frequency and percentages.

RESULTS

Of the 150 total NS1-positive patients, 26(17%) were also positive for anti-IgM. Among the 150 NS1-positive cases, 99(66%) were males, 13(13%) were Dengue anti-IgM positive, 51(34%) were females, and 13(25%) were Dengue anti-IgM positive (Table-I).

Table-I: Frequency Distribution of Dengue anti-IgM in NS1 Cases (n=150)

		Total NS1 cases (n=150)	Dengue anti-IgM Status	
Gender	Male	99(66%)	13(13%)	86(87%)
	Female	51(34%)	13(25%)	38(75%)

Among age groups distribution, the highest frequency was found in the age group (26-45 years), which had 60 NS1 positive cases, out of which 12 were anti-IgM positive, and the least prevalence was showed by age group (66-85 years), which had only 9 NS1 positive cases, of which two were anti-IgM positive (Figure). Signs and symptoms observed in patients were fever, headache, body aches, and retro-

orbital pain. 150 (100%) patients suffered from fever, 140 (93%) had headache, 120(80%) had body aches, and 94(63%) had retro-orbital pain.

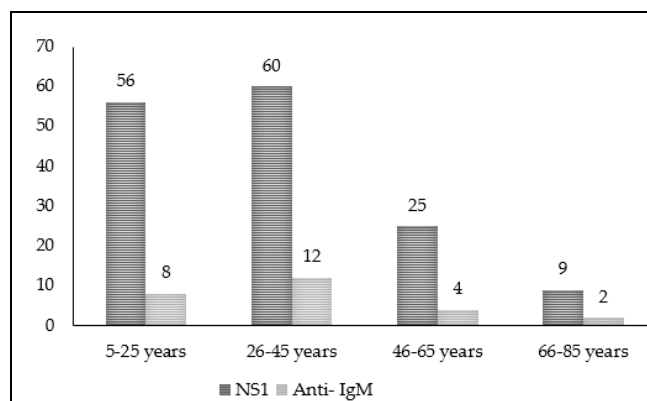


Figure: Dengue anti-IgM in NS1 Positive Cases Among Age Groups (n=150)

One hundred and fifty NS1-positive samples were collected on different symptoms' onset days and tested for anti-IgM to determine their association. Out of 150 NS1 positive cases, 54 and 44 samples were collected on days 1 and 2 of onset of symptoms, respectively, but no sample was found positive for anti-IgM. On day 3, 18 samples were collected, of which 5(27%) were anti-IgM positive. On day 4, 13 samples were collected, of which 7 (53%) were anti-IgM positive. On day 5, 9 samples were collected, of which 5(55%) were anti-IgM positive. On day 6, 5 samples were collected, of which 3(60%) were anti-IgM positive. On day 7, 4 samples were collected, of which 3(75%) were anti-IgM positive. On day 8, 3 samples were collected, and anti-IgM positivity was 100% (Table-II).

Table-II: Frequency on Basis on Day of Onset of Symptoms (n=150)

	Day of onset of symptoms	Total NS1 cases	Dengue anti-IgM status	
			Positive	Negative
	1	54	0	54(100%)
	2	44	0	44(100%)
	3	18	5(27%)	13(73%)
	4	13	7(53%)	6(47%)
	5	9	5(55%)	4(45%)
	6	5	3(60%)	2(40%)
	7	4	3(75%)	1(25%)
	8	3	3(100%)	0
Total		150	26(17%)	124(83%)

DISCUSSION

According to researchers, no outbreaks were reported until 1994 in Pakistan due to a lack of surveillance and diagnosis. The first outbreak of

Dengue in Pakistan started in August 1994 and continued through November 1994 in Karachi, leading to morbidity in the thousands.¹² It significantly decreased after 1950 and returned to Pakistan in the 1980s in two episodes in the south and 1993 in the north.¹³ Many studies confirmed the serotypes in all these outbreaks, and serotypes 1 and 2 were found.¹⁴ In 2005, another serotype, three, was reported in Pakistan after a break of 10 years.^{15,16}

A prospective hospital-based observational study was conducted in 2019 at Lady Reading Hospital in Peshawar, Pakistan. A total of 415 dengue-positive patients (309 males and 106 females) were included in the study. The highest prevalence (38%) was seen in the age group 21-40 years, while (21%) was in the age group 1-20 years. Fever was recorded in 100% of dengue patients, while 73% of patients showed symptoms of headache and fatigue, and 70% had liver abnormalities.⁸

In 2020, a clinical cohort study was conducted in the USA to check the persistence of symptoms in confirmed dengue patients by following them up to 6 months. The outcomes were a rapid decline in signs of pain after the eighth day from the onset of fever. Some patient's pain signs might last up to 60 days, particularly headaches, myalgia and arthralgia. Gastrointestinal symptoms (hyperoxia and nausea) slightly increased between 30-60 days. Some patients (21-25%) experienced dizziness when standing up from 8-120 days after onset of fever. The most persistent symptoms of eye illness were blurred vision and phosphene for 15-30 days in 17.7% and 10%.¹⁷

In our study, 150 dengue NS1 positive patients were included. The highest frequency (40%) of dengue NS-1 antigen was observed in the age group (26-45 years). Among this age group (26-45 years), 20% were anti-IgM positive. The lowest prevalence (6%) of dengue NS1 antigen was observed in the age group (66-85 years). Among this age group (66-85 years), 22% were anti-IgM positive. 100% of patients in this study showed fever symptoms, 93% showed headache, 80% had body aches, and 63% faced retro-orbital pain.

In 2012, a review article entitled "Diagnosis of dengue" revealed that DENV is detected by the highly conserved glycoprotein dengue NS1, secreted from infected cells as a hexamer. It can be found up to 9 days in peripheral blood circulation and can persist for up to 18 days from the onset of illness in some cases. Dengue anti-IgM can be detected 3-5 days after the onset of illness and may persist for up to 2 weeks.¹⁸ In

2013, a prospective observational study was conducted in Swat and Mansehra, Pakistan, to test the samples to detect NS1 antigen and anti-DENV IgM antibody by ELISA. A total of 323 samples were collected on different days. On days 1-3, 71 samples were collected, of which 57(80%) were positive for NS1 and 25(35%) were positive for anti-DENV IgM antibody. On days 4-6, 170 samples were collected, of which 115 (68%) were positive for NS1 and 102(60%) for IgM. On days 7-8, 63 samples were collected, of which 30(48%) were positive for NS1 and 48(76%) for IgM. On day 9, 19 samples were collected. Among those, 7(37%) were positive for NS1 and 15(79%) were positive for IgM.¹⁹

In 2010, a study was conducted in Finland to diagnose dengue infection in travellers. Dengue NS1 antigen was positive in 84.2% of the samples collected on days 6-7, while NS1 antigen positivity was slightly low in the samples collected on days 1-3 (78.6%) and 4-5(74.1%). The positivity of anti-IgM was also tested based on the onset of symptoms; on days 1-3, the positivity of anti-IgM was 42.9%. On days 4-5, the positivity was 81.5%; on days 6-7, it was 94.7%.²⁰

According to our study, samples were collected on different days to test anti-IgM positivity in NS1-positive samples. A number of days were counted after the onset of symptoms. On day 1 and 2, no sample was positive for anti-IgM. Anti-IgM started appearing on day 3(27%), which increased gradually as the time of infection increased. On day 4, it was 53%; on day 5, it was 55%; on day 6, it was 60%; on day 7, it was 75%, and on day 8, it was 100%. So, it depicts that Dengue anti-IgM showed less or no positive in the initial days of illness, while its positivity increased as the number of days of illness increased. Moreover, the Dengue anti-IgM positivity was low compared to Dengue NS1 antigen, i.e. 17% of the total 150 NS1 positive samples, so it was a non-significant parameter. Although a sufficient amount of data is available on determining the association of Dengue anti-IgM and NS1 antigen with respect to the onset of symptoms, no such study was found in the 2021 outbreak. This helps to reduce the study gap. This data would be beneficial for clinicians and researchers in recognition of the association of acute Dengue markers with respect to the onset of symptoms to counter this emerging disease.

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LIMITATION OF STUDY

The limitations of our study was that the duration was short, which could have affected the results.

CONCLUSION

The present study's findings suggest that there is great significance of serological markers, i.e. Dengue NS1 and anti-IgM, in the diagnosis of stage of acute Dengue infection.

Conflict of Interest: None.

Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

SKN & AB: Data acquisition, data analysis, data interpretation, critical review, approval of the final version to be published.

EG & MAR: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

HH & HH: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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