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Original Research Paper

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SERO-PREVALENCE OF DENGUE VIRAL INFECTION IN RAJOURI DISTRICT OF JAMMU AND KASHMIR

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ABSTRACT Rajouri district is an endemic area, with an increasing number of febrile illness cases in our hospital every year. In this retrospective study a total of 543 blood samples collected from clinically suspected cases of dengue viral infection were analyzed for NS1 Ag and IgM antibodies for a period of 2 years. Serum of the patients having fever < 5 days was put to detection of NS1 antigen and patients having fever > 5 days was subjected to serological assay of anti-dengue IgM antibody. 198 (52.11%) of the suspected cases were found Seropositive. NS1 Ag ELISA was positive in 154 (39.58%) samples out of the total 389 and IgM antibody capture ELISA was positive in 44(28.57%) samples out of the total 154. Seropositive cases were more in males (62.41%) and in the age group 21-30 yrs (32.21%). Fever 171 (57.38%), headache 150 (50.33%) and vomiting 131 (43.95%) were the most common clinical manifestations. Timely detection of NS1 antigen ELISA and IgM antibody ELISA can be very useful in early diagnosis of patients, identification of patients at risk of hemorrhage, dengue management and control of dengue viral infection.

KEYWORDS : Antibody, serological assay, ELISA.

Dengue is the most rapidly spreading mosquito-borne viral disease, with a 30-fold increase in global incidence over the past five decades. The global incidence of dengue fever is on the rise since the year 2000 due to factors like rapid urbanization, expanding human population, increased global travel and geographical expansion of the primary vector, Aedes aegypti. The globally estimated burden of symptomatic cases ranges to 96 million and 58.4 million cases/ year. Dengue has been known to be endemic in India for over two centuries. Among 18 endemic states, the most affected regions are Delhi, West Bengal, Kerala, Tamil Nadu, Karnataka, Maharashtra, Rajasthan, Gujarat and Haryana.[1]

The etiological agent is five serotypes of dengue virus (DV) namely DEN-1, DEN-2, DEN-3, DEN-4 and DEN-5 belonging to genus Flavivirus and family Flaviviridae. The primary vectors for its spread are infected *Aedes aegypti* and *Aedes albopictus* mosquito species. Dengue virus causes a broad spectrum of illness ranging from mild asymptomatic illness to severe Dengue Haemorrhagic Fever/ Dengue Shock Syndrome (DHF/DSS).[2]

There is no prevention in the form of any vaccine for dengue, thus early confirmed diagnosis and treatment is recommended for preventing complications and disease control in the region. Diagnosis of Dengue infection may be made by various methods which include viral isolation in cell culture, viral nucleic acid detection (RT-PCR), immunofluoroscence or by detection of viral RNA by nucleic acid amplification tests (NAAT). These methods require expertise, expensive equipments & reagents and there is time delay.[3]

Serological test which detects dengue specific IgM immunoglobulin and IgG immunoglobulin by ELISA are the most commonly used methods. Dengue virus (DENV) nonstructural protein 1 (NS1) Ag detection is also important serologic method for early diagnosis of Dengue infection. Dengue NS1 antigenemia is detectable within 24 hrs and may persist for nine days following the onset of illness. IgM antibodies level increases rapidly and appears to peak about 2 weeks after the onset of symptoms, then decreases to undetectable levels over 2–3 months.[4]

As Rajouri district, is an endemic area, there is an increasing number of febrile illness cases in our hospital. Thus, the present study is the first study in Rajouri district that was under taken to know the prevalence of dengue virus infection by ELISA methods (IgM capture ELISA, NS1 Ag direct sandwich ELISA) along with clinical correlation that was used for laboratory diagnosis of dengue.

MATERIAL AND METHODS

This was a retrospective study conducted in the Department of Microbiology, Government Medical College, Rajouri over a period of 2 years i.e. from January 2018- December 2019. A total of 543 blood samples were collected from clinically suspected cases of dengue viral infection referred from various OPDs, IPDs and Emergency services in our hospital.

INCLUSION CRITERIA

Patients suspected of Dengue viral fever with two or more of the following symptoms like headache, rash, retro-orbital pain, myalgia, arthralgia, hemorrhagic manifestations and leucopenia.

EXCLUSION CRITERIA

Unlabelled, haemolysed, and lipaemic blood samples were excluded.

Sample Collection

A single blood sample (approximately 2-3 ml) was collected from each patient suspected of Dengue viral infection. Details of the patients were recorded which included age, sex and address of the patient, clinical details, platelet count, date of blood collection post onset of illness. After collection, samples were allowed to clot at room temperature and then serum were separated and processed immediately. In case of delay in processing, they were stored in refrigerator at a temp of 2- 8°C for 48 hrs.

Sample Processing

Dengue NS1 antigen detection:
Serum of the patients having fever < 5 days was put to detection of NS1 antigen using Merilisa Dengue NS1 Ag ELISA kit. The test is based on sandwich format microplate enzyme immunoassay with monoclonal anti dengue NS1 antibodies coated microtitre plates for the detection of dengue virus NS1 antigen in human serum. The test was performed as per the manufacturer's instructions.

Anti-Dengue IgM antibody detection:
Sera of patients having fever > 5 days was subjected to serological assay of anti-dengue IgM antibody present in samples and it was carried out using NIV dengue IgM capture ELISA kit. IgM antibodies in the patient's serum are captured by anti-human IgM coated on the solid surface. Dengue Ag was added which binds to captured human IgM in the sample. The results were read and interpreted as per the manufacturer's instructions.

RESULTS

In this study a total of 543 clinically suspected samples of dengue viral infection were tested for NS1 Antigen and IgM Antibodies. 198 (52.11%) of the suspected cases were found seropositive for Dengue between 2018- 2019.

389 samples were subjected to NS1 Ag ELISA which reported 154 (39.58%) samples as positive and rest 235 (60.41%) were negative for the same. On the other hand IgM antibody capture ELISA was positive in 44(28.57%) samples out of the total 154. **(Table 1)**

Table 01: Comparison of results of samples tested by NS1 Antigen ELISA and IgM Antibody detection test:

	Positive	Negative	Total
NS1 Antigen ELISA	154 (39.58%)	235 (60.4%)	389
IgM capture ELISA	44 (28.57%)	110 (71.42%)	154

Of the 298 sero-positive cases, 186 (62.41%) were male and 112 (37.58%) were female. **(Graph 1)** Number of patients suffering from dengue infection were more in the age group 21-30 yrs (32.21%) followed by 10-20 yrs (25.50%) and 31-40 yrs (21.81%). **(Table 2)**

Table 02: Age-wise distribution of Sero-Positive Cases:

Age-group	N	%age
<10 yrs	5	(1.6%)
10-20 yrs	76	(25.50%)
21-30 yrs	96	(32.21%)
31-40 yrs	65	(21.81%)
41-50 yrs	31	(10.40%)
51-60 yrs	15	(5.03%)
>60 yrs	10	(3.35%)
Total	**298**	**100%**

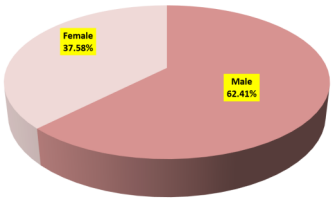
Seasonal variation depicted that spike in number of dengue suspected cases were in the months of October 329 (60.5%), November 119 (21.91%) and September 65(11.97%). **(Graph 2)**

Clinical presentations in Dengue positive cases depicted that fever 171 (57.38%), headache 150 (50.33%) and vomiting 131 (43.95%) were the most common manifestations. **(Table 3)**

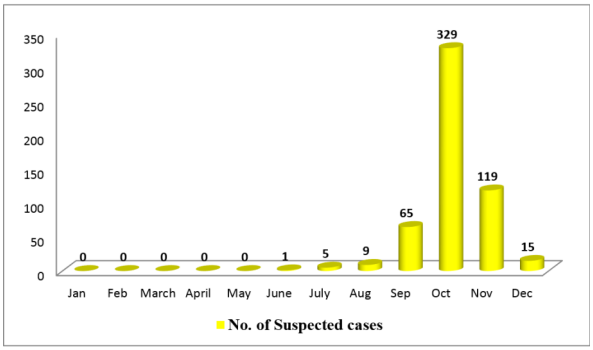
Table 03: Major clinical manifestations of Dengue Positive Cases:

Clinical Manifestation	N	%age
Fever	171	57.38%
Arthralgia	98	32.88%
Hemorrhagic Manifestations	55	18.45%
Vomiting	131	43.95%
Abdominal pain	43	14.42%
Headache	150	50.33%

Graph 01: Gender wise distribution of Seropositive cases.



Graph 2: Month-wise distribution of Dengue Suspected Cases (Seasonal Variation)



DISCUSSION

There has been an annual surge in Dengue cases in India. Thus there arises a need for specific, inexpensive dengue diagnostic tests that can be used for clinical management, surveillance and would permit early intervention to treat patients and prevent or control epidemics. The National Vector Borne Disease Control Programme and Integrated Disease Surveillance Programme (IDSP) are mutually functioning for passive sentinel surveillance program for prevention and control of dengue.[5]

ELISA has become the most powerful assay for serodiagnosis due to its high sensitivity (99.5%) and specificity (100%) in the case of NS1 Ag MICROELISA and sensitivity (99.13%) and specificity (99.84%) for IgM/ IgG MICROELISA.[1] In the present study, 52.11% of the suspected cases were found seropositive for dengue between 2018- 2019. Similar results were found in study conducted by Tahir et al. This may be due to the spread of the disease from urban to suburban and rural areas the actual number of cases may count in millions.

NS1 Ag ELISA and IgM antibody capture ELISA reported 39.58% and 28.57% samples as positive. Dengue NS1 antigen has allowed for early detection of DENV infection as the antigens remain detectable in blood for 5 days after onset of fever and rapidly disappears after formation of specific antibodies. IgM antibodies appear 7-10 days following dengue infection and remain for many months.[7]

The seropositive cases were more in males in comparison with females and the age group in which the cases showed predominance was between 21 to 30 yrs. (32.21%). These findings were similar to studies conducted by Tabasum et al [8] and Sarah et al [9]. It might be due to differences in sociocultural environment where male are more exposed to outdoor activities and their bodies less covered as compared to females.

The prevalence was more in months of October, November and September following the rains after which there was decline in Dengue fever cases. The findings were in concordance with study conducted by Chandran et al [10]. The reason for this can be the heavy rains of monsoon season, which usually start in August, September resulting in stagnant water that serves as breeding ground for vectors of this virus. Also the breeding habit of Aedes aegyptis is highest during post monsoon period.[11]

In the present study, the most common symptoms among the total study population were fever (57.38%), headache (50.33%) and vomiting (43.95%). Similar findings were found in study conducted by Archana Nagarajan et al [12] and Mishra et al [13] where fever and headache was the most common manifestations of dengue fever. These clinical findings can help the physician in presumptive diagnosis of Dengue and take appropriate steps in patient management.

CONCLUSION

Dengue being a persistent viral infection in the Indian subcontinent with increasing incidences in mortality.This study concludes that timely detection of NS1 antigen ELISA and IgM antibody ELISA act as highly sensitive and specific tools which can be very useful in early diagnosis of patients, identification of patients at risk of hemorrhage, dengue management and control of dengue viral infection. This can definitely help resource-limited countries like India which experiences dengue outbreaks annually and it is a seasonal trend. Serological tests can be used in the laboratories that have limited resources, lack viral culture or RT-PCR facilities.

Conflicts of Interest

"The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper."

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