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# Serodiagnostic and Clinical Presentation of Dengue Virus Infection in A Tertiary Care Hospital

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**The scale and intensity of present outbreak confirms that a new paradigm of Dengue disease, a complex in the form of Dengue Hemorrhagic Fever and Dengue Shock Syndrome has now secured a firm foothold in India. The new lethal manifestation of an old benign disease broke out in the Central India. The objective of the study was to evaluate the utility of serodiagnosis of Dengue virus infection and correlate the serological results with clinical presentations in patients diagnosed to be suffering from dengue at a tertiary care hospital in Indore, Madhya Pradesh, Central India. Laboratory diagnosis of Dengue Virus infection mainly depends on detection of Antigen or virus specific Antibodies. The patients admitted with Dengue-like symptoms were screened for their seropositivity by Rapid Immunochromatographic and ELISA methods and the results were correlated clinically. Out of the 585 patients tested, 317 were seropositive. About 46.37% patients tested positive for Dengue NS1 Antigen and 57.41% patients tested positive for IgM antibody. The patients presented with a range of signs and symptoms indicative of different phases of disease. Hundred percent patients categorized under DHF and DSS were found to be in the acute phase of infection. Most of the reported cases occurred in young adults. Hence it was concluded that rapid, accurate and early serodiagnosis of dengue virus infection from acute phase viremic blood samples from patients in febrile stage contributes greatly to patient management in hospitals and control measures in public health.**

**Key words:** Dengue Hemorrhagic Fever, Dengue Shock Syndrome, Dengue Antigen, Dengue IgM.

Dengue or Break Bone Fever is endemic to Indian subcontinent[1] since last two centuries, as a benign and self- limited disease. But in recent years it has changed its course manifesting in severe form as Dengue Hemorrhagic Fever [DHF] and Dengue Shock Syndrome [DSS]. *Aedes aegypti*[2, 3] is a formidable foe and an efficient vector while humans are the amplifying hosts of the virus[4] causing this acute febrile illness. The problem is acute in developing countries, where inadequate utilities force residents to store water in containers and tanks, prime breeding grounds for vectors. Early diagnosis of dengue is important and can be established with commercially available serological assays. Early case detection and management reduce morbidity and mortality due to DHF and DSS.

## MATERIAL AND METHODS

During the period between August'2009 and October'2009, there has been an outbreak of dengue in the city of Indore and surrounding districts, in the Malwa region of the state of Madhya Pradesh, Central India. Six hundred and thirty five patients suspected to be suffering from dengue virus infection were admitted in our institute. Five hundred and eighty five patients were tested for

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Dengue NS1 antigen and Dengue antibodies[IgG/IgM] by an immunochromatographic method[5][*SD Bioline Dengue Duo from Standard Diagnostics,Inc.Sensitivity for Ag 92.8%,for IgG/IgM 99.4%;Specificity for Ag 98.4% , for IgG/IgM 93%*] and ELISA method[6][*Dengue Ag(NS1Ag) Platelia™ Dengue NS1 Ag from BioRad;Sensitivity in IgG Negative sample is 98.5% and for IgG positive sample is 85.6%,Specificity 100%; Dengue IgG/IgM Microlisa from J.Mitra & Co.pvt. Ltd.,For IgM Sensitivity 96.15%,Specificity 99.84%;For IgG Sensitivity 95.55%,Specificity 99.90%*] Fifty patients were not serodiagonised. Their treatment was started on the basis of their clinical symptoms and hematological tests. A total of 317 out of 585 patients [54.18 %] tested positive for either Ag or Ab [IgG/IgM] or both Ag and Ab.

The Case records of 317/585 seropositive patients were analyzed for clinical and laboratory data. As per the WHO classification, the proposed probable diagnosis-an acute febrile illness with two or more of the following manifestations, headache, retro-orbital pain, arthralgia, rash, haemorrhagic manifestations, leucopenia and a positive IgM antibody test on serum samples collected five or more days after the onset of fever supports the diagnosis of dengue[7].The study was approved by the ethical committee of our Institute.

## RESULTS

Three hundred and seventeen of the 585 seropositive cases of dengue were observed for their clinical and laboratory features. The age of these patients ranged between 9 and 81 years. The

**Table 1.** Serodiagnosis results of patients presenting seropositivity.

| | Total No. of Patients | Antigen Positive | Antibody Positive | | Antigen & Antibody Positive | | | |
|---|---|---|---|---|---|---|---|---|
| | | | IgM | IgG | IgM+IgG | Ag+IgM | Ag+IgG | Ag+IgM+IgG |
| DF | 300 | 45 | 21 | 75 | 65 | 49 | 11 | 34 |
| DHF | 10 | 03 | 05 | - | - | 02 | - | - |
| DSS | 07 | 01 | 04 | - | - | 02 | - | - |

**Table 2.** Clinical manifestations of patients with dengue fever[n=300]

| Characteristic | Numbers | Percentage |
|---|---|---|
| Mild Febrile Syndrome | 300 | 100 |
| Nausea &Vomiting | 236 | 78.6 |
| Severe Headache | 144 | 48 |
| Cough | 136 | 45.3 |
| Myalgia | 120 | 40 |
| Thrombocytopenia | 104 | 34.6 |
| Rashes | 40 | 13.3 |
| Constipation | 32 | 10.6 |
| Retro-orbital Pain | 32 | 10.6 |
| Dehydration | 32 | 10.6 |
| Pain in Chest | 28 | 9.3 |
| Decreased Appetite | 28 | 9.3 |
| Abdominal Pain | 24 | 08 |
| Itching | 20 | 6.6 |
| Hemorrhagic Manifestations | 20 | 6.6 |
| Leucopenia | 20 | 6.6 |
| Vertigo | 16 | 5.3 |
| Irritability | 12 | 04 |
| Lethargy | 08 | 2.6 |
| Throat Pain | 08 | 2.6 |

**Table 3.** Signs and Symptoms presented by patients with DHF [n=10]

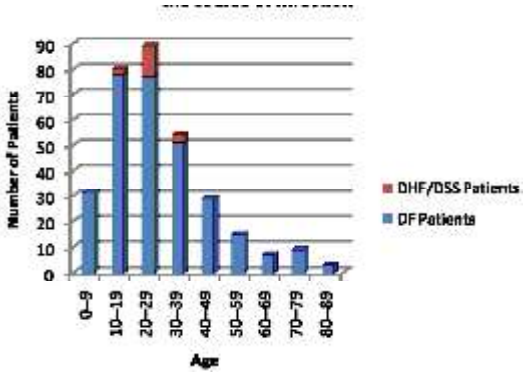
| Characteristic | Numbers | Percentage |
|---|---|---|
| High Fever of Abrupt Onset | 10 | 100 |
| Bleeding Tendencies | 10 | 100 |
| Capillary Plasma Leakage as evidenced by Hematocrit | 09 | 90 |
| Hepatomegaly | 05 | 50 |
| Pleural Effusion | 05 | 50 |

**Table 4.** Signs and Symptoms presented by patients with DHF [n=7]

| Characteristic | Numbers | Percentage |
|---|---|---|
| Hypotension | 07 | 100 |
| Weak pulse | 07 | 100 |
| Restlessness | 07 | 100 |

most affected age group was between 9 and 39 years. The patients who progressed to DHF and DSS also belonged to this particular age group [Fig.1]

Out of 317 seropositive patients, 57.41% patients tested positive for IgM and 46.37% patients tested positive for Ag [Table 1].




Data used to plot the graphs mentioned in the manuscript

Fig.1. Age profile of dengue patients during the course of infection

| Age Group[yrs.] | Total No.of Patients | |
|---|---|---|
| | DF | DHF |
| 0-9 | 31 | 00 |
| 10-19 | 78 | 02 |
| 20-29 | 77 | 12 |
| 30-39 | 51 | 03 |
| 40-49 | 29 | 00 |
| 50-59 | 15 | 00 |
| 60-69 | 07 | 00 |
| 70-79 | 09 | 00 |
| 80-89 | 03 | 00 |

**Fig. 1.** Age profile graph of dengue patients during the course of infection

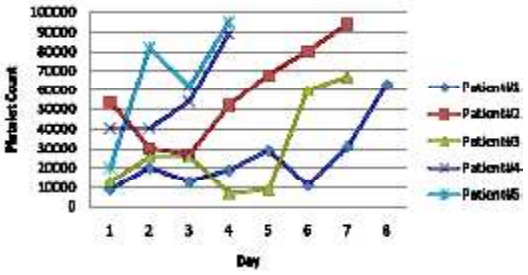



**Fig. 2(a).** Platelet Count of DHF patients during the course of Infection[n=5]

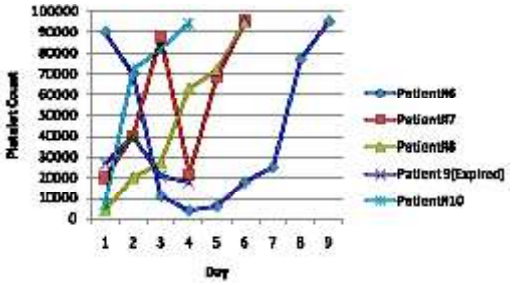



**Fig. 2(b).** Platelet Count of DHF patients during the course of Infection[n=5]

Data used to plot the graphs mentioned in the manuscript

**Fig. 2.** [a, b] Platelet Count of DHF patients during the course of Infection

| Day | Patient#1 | Patient#2 | Patient#3 | Patient#4 | Patient#5 | Patient#6 | Patient#7 | Patient#8 | Patient#9 | Patient#10 |
|---|---|---|---|---|---|---|---|---|---|---|
| 01 | 9000 | 54000 | 13000 | 40000 | 20000 | 90000 | 20000 | 4000 | 27000 | 7000 |
| 02 | 20000 | 30000 | 26000 | 40000 | 82000 | 70000 | 40000 | 20000 | 40000 | 72000 |
| 03 | 13000 | 27000 | 26000 | 54000 | 62000 | 11000 | 88000 | 27000 | 21000 | 82000 |
| 04 | 19000 | 52000 | 7000 | 89000 | 95000 | 4000 | 22000 | 63000 | 18000 | 94000 |
| 05 | 29000 | 68000 | 9000 | | | 6000 | 69000 | 72000 | | |
| 06 | 11000 | 80000 | 60000 | | | 18000 | 95000 | 95000 | | |
| 07 | 31000 | 94000 | 67000 | | | 25000 | | | | |
| 08 | 63000 | | | | | 77000 | | | | |
| 09 | | | | | | 95000 | | | | |

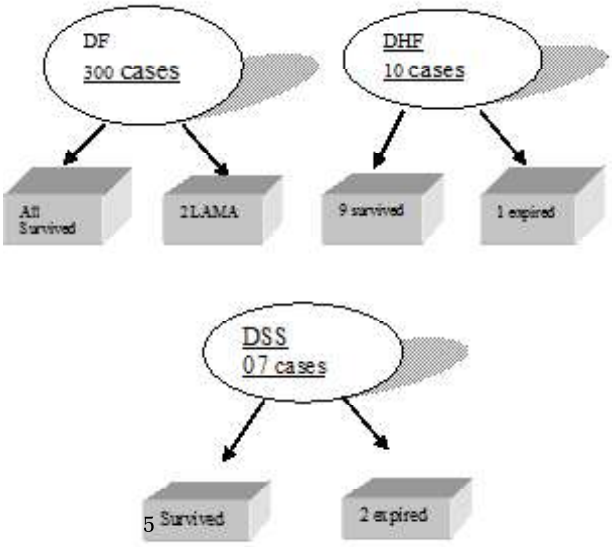



**Fig. 3.** Outcome of Dengue Infection

The majority clinical findings in the reported DF cases were febrile illness, thrombocytopenia, severe headache, nausea and vomiting[8] (Table 2).

In the ten patients, out of 317 who progressed to DHF, the major clinical findings were abrupt onset of high fever, bleeding tendencies, hepatomegaly and capillary plasma leakage[7] (Table 3).

The ten DHF patients exhibiting severe thrombocytopenia and the pattern of their platelet counts during the course of infection has been graphically represented (Fig. 2 a, b) Each series represents a single patient.

Seven patients out of 317 progressed to DSS.They had persistent hypotension and weak pulse, restlessness, cold and clammy skin. (Table 4).

## DISCUSSION

The epidemiology of dengue in the Malwa region of Madhya Pradesh showed that most reported cases occurred in young adults. Among the clinical signs a large percentage of people with dengue fever presented with febrile syndrome, severe nausea, vomiting, myalgia, rashes and thrombocytopenia. While, DHF is a biphasic febrile illness, with hemorrhagic tendencies due to increased vascular permeability and plasma leakage[7]. The dengue patients especially the DHF patients exhibited a severe thrombocytopenia during the course of disease and this could very well be managed through transfusion therapy. By the time the patients recovered, they had attained a normal platelet count. Laboratory criteria for confirmation of dengue fever are isolation of dengue virus from serum and detection of dengue virus genomic sequences by polymerase chain reaction[9]. However these tests are only available in reference laboratories and not available routinely. Hence, serological tests that demonstrate either a dengue antigen or IgM antibody to one or more dengue virus antigens in the serum have greatly increased our ability effectively and efficiently diagnose acute dengue infections.

Diagnosis of dengue infection during the febrile stage has been challenging. On correlating the serological results with the clinical types of presentation of Dengue among the study patients, 225/300(75%) DF patients and 17/17(100%) patients with DHF & DSS had a primary Ab response or presented with antigen positivity. In numerous acute dengue fever patients, an early diagnosis can be obtained only by combining IgM Ab

detection with detection of virus. In our study, dengue specific IgM antibodies were positive in 57.41% of the acute phase sera which is comparable with 52.3% seropositivity found in 2003 outbreak in Delhi and 43% seropositivity found in 2003 epidemic in and around Lucknow.[10]

While all the 300 patients with uncomplicated DF survived, 1/10(10%) patients with DHF & 2/7(28.57%) patients with DSS expired [Fig.3] All the three expired patients were in acute phase of infection.

The clinical observations and serological test profile can be interpreted by the fact that most of the DHF patients were in the state of fever since last 5-6 days and IgM seemed to present predominantly in their serum. Out of the 7 patients who progressed to DSS, 2 presented with some complications like Encephalitis and the other three presented with severe chest pain and pleural effusions along with the other clinical signs commonly manifested by DSS patients. Two of the 7 of the DSS patients expired. Firstly, they were presented to the hospital lately. Secondly, they had multiple complications and inspite of close monitoring and best treatment given to them, they succumbed to death. As such the overall survival rate was high. This can be maintained by early serodiagnosis and proper conservative management to decrease the incidents of death. In our study, early serodiagnosis and admission of suspected dengue cases and prompt administration of intravenous fluids to individuals showing signs of premonitory vascular collapse have brought case fatality rates down sharply in dengue endemic areas. We believe that serodiagnosis has a role in picking up severely ill patients and preventing them from entering into more severe phases of diseases.

## ACKNOWLEDGMENTS

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