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A Serological Study of Dengue and Hanta Virus in Acute Febrile Patients in a Tertiary Care Hospital

[1,]Tejashree.A , [2,]Thejaswini.H.S, [3,]Madhuri Kulkarni.
Department of Microbiology, JSS Medical College, Mysore- 570004, Karnataka, India.

ABSTRACT : *The emerging viral diseases Haemorrhagic Fever with Renal Syndrome (HFRS) and Hanta virus Cardiopulmonary Syndrome (HCPS) are a cause of global concern as they are increasingly reported from newer regions of the world. Dengue also called classic dengue or break bone fever is a Flaviviral infection found in large areas of tropical & subtropical region. Clinically, the febrile illness produced by Dengue virus, Leptospira serotype & Hanta virus may be indistinguishable.*

MATERIALS AND METHODS: *A total of 1782 serum samples were tested from febrile patients for Dengue NS1 Ag, Dengue IgG/IgM by Immunochromatographic Test (ICT) kit & 100 randomly selected serum samples were tested for Hanta virus IgM/IgG ICT by SD Bioline. Other investigations included Hemoglobin estimation, Total & Differential Leucocytes count, hematocrit, platelet count and Liver Function Test (LFT).*

RESULTS: *Out of 1782 serum samples tested, 79 patients (4.4%) tested positive for dengue serology. None of the samples tested positive for Hanta virus IgG/IgM. To conclude, ICT helps for rapid diagnosis of Dengue & Hanta virus infection.*

KEYWORDS: *Dengue fever & Hanta virus, Serology, ICT*

I. INTRODUCTION:

Hanta viruses are enveloped viruses with negative sense single stranded RNA genome and belong to the family Bunyaviridae[1]. The emerging viral diseases Hemorrhagic fever with renal syndrome (HFRS) and Hantavirus cardiopulmonary syndrome (HCPS) are a cause of global concern as they are increasingly reported from newer regions of the world. Humans are accidental hosts and get infected by aerosols generated from contaminated urine, faeces and saliva of infected rodents. The first Hantavirus isolate to be cultured, Thottapalayam virus, is the only indigenous isolate from India, isolated from an insectivore in 1964 in Vellore, South India. Research on Hantavirus in India has been slow but steady since 2005[2]. Serological investigations of patients with febrile illness revealed presence of anti-Hantavirus IgM antibodies in 14.7% of them. The seropositivity of Hantavirus infections in the general population is about 4% and people who live and work in close proximity with rodents have a greater risk of acquiring Hantavirus infections. Hantavirus infection can appear clinically uncharacteristic and may mimic other syndromes[3].This compounds the difficulties in diagnosing Hantavirus infections in areas where the disease is not endemic and clinical cases may be sporadic[4]. Further, less pathogenic Hantaviruses may cause a greater amount of asymptomatic infections, as seen for HFRS in Europe and Asia[5].

Dengue, also called classic dengue or break bone fever is a Flaviviral infection found in large areas of tropical and subtropical regions[6]. Dengue is a mosquito borne viral infection and is transmitted by Aedes aegypti and Aedes albopictus[7,8]. Four distinct serotypes of viruses DEN-1, DEN2, DEN3, and DEN-4 cause dengue[8,9]. Humans are the main amplifying hosts of virus. Dengue may be asymptomatic or may lead to undifferentiated fever, dengue fever (DF) or dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). DHF and DSS are leading causes of hospitalization and death especially among children[8,10]. Dengue is the most important of the Arboviral infections of humans[8]. In India, epidemics are becoming more frequent[11,12]. Involvement of younger age group and increase in the frequency of epidemics are indicators of higher incidence of infection[11]. If untreated, mortality from complications of Dengue fever is as high as 20%, whereas if recognized early and managed properly, mortality is less than 1%[12]. Early diagnosis is essential and clinical suspicion is based on the frequency of symptoms in the population[13].

Clinically, the febrile illness produced by dengue virus, leptospira serotypes and hanta viruses may be indistinguishable[14]. Mysore experienced an outbreak of dengue recently. Even now several cases of thrombocytopenia are being detected daily by the clinicians.

In this regard, an attempt is being made to detect the number of Dengue/Hanta virus cases in this region by using rapid Immunochromatographic test which detects IgG/IgM antibodies for Dengue and Hanta virus and NS1 antigen for Dengue.

II. MATERIALS AND METHODS:

This study was carried out in the Department of Microbiology, JSS Hospital, Mysore from Jan 2012 to Dec 2012 when there was an outbreak of Dengue fever. A total of 1782 serum samples were collected from febrile patients. 100 random samples, which were Negative for Dengue antibodies were tested for IgG/IgM antibodies to Hantavirus by ICT. The serum samples were tested for Dengue NS1 Ag, Dengue IgG/IgM Ab by ICT kit SD-Bioline. The study group included children and adults presenting with fever, headache, vomiting and pain abdomen. Laboratory investigations were carried out in these patients for Hemoglobin, total & differential leucocyte count, hematocrit, platelet count and LFT.

III. RESULTS:

A total of 1782 serum sample were tested for Dengue IgG/IgM, NS1 Ag and 100 randomly selected serum samples were tested for Hantavirus IgG/IgM by rapid ICT (SD-Bioline), 79 patients (4.4%) were positive for Dengue serology. Among 79 patients, only 52 cases could be reviewed. Out of 52 patients, 34(65.38%) were male and 18(34.6%) were female. Peak incidence of Dengue fever was found in the age group of 21-40 years (39%). Table-1 shows the demographic profile. 20 of them tested positive for NS1 Ag, 11 of them had both IgG and IgM as shown in Table-3. Out of 52 cases reviewed, 43(49%) of them had associated thrombocytopenia. 6 had severe thrombocytopenia with platelet count <20,000/mm^3 and had bleeding tendency. Frank bleeding (Haematemesis) was seen in 2 cases. On clinical examination, the most common finding was Hepatomegaly. Other findings included splenomegaly and 16 of them had both Hepatomegaly and splenomegaly. 9 of them had epigastric tenderness. Other laboratory investigations revealed that a large population had mild anaemia and liver enzymes were elevated in 30% of them who were seropositive.

None of the sample tested positive for Hanta virus IgG/IgM.

IV. DISCUSSION:

This is probably the first study in Karnataka performing the serological test of hanta virus. Clinically the febrile illness produced by Dengue viruses, Leptospira serotypes and Hanta viruses may be indistinguishable[10].

As per 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 positive IgM Ab test on serum samples collected five or more days after the onset of fever supports the diagnosis of Dengue fever[15].

In our study, Dengue NS1 Ag, IgG/IgM Ab were detected by ICT by SD Bioline rapid kit test and Hanta virus IgG/IgM by SD Bioline rapid (ICT) kit test.

A total of 1782 serum sample were tested for Dengue virus and 100 randomly selected serum sample for Hanta virus. 79 patients (4.4%) were seropositive for Dengue virus, only 52 could be reviewed . A study conducted by A.Chakravarti et.al showed 25% incidence of Dengue seropositivity[16].

In the present study, among 52 dengue seropositive cases, 20 of them tested positive only for NS1 Ag & 11 of them had both IgG/IgM Ab for Dengue virus.

Detection of NS1 Ag is useful in the diagnosis of early stage of Dengue virus infection. The combined use of IgG and IgM is an alternate strategy for the diagnosis of dengue virus infection, since sensitivity for secondary dengue is improved and specificity for other infection is also improved[17].

In our study primary infection was detected in 20 patients and secondary infection in 11 patients. Higher incidence of Dengue fever was seen in male patients (65.38%) than in female (34.6%). In this study Dengue fever was common in the age group of 21-40years (39%) followed by 0-20years (35%).

Early in the infection, it may be difficult to differentiate DHF from other febrile illness. Later usually after 3 or 4 days, when thrombocytopenia and hemoconcentration are present DHF is easier to diagnose. Among these 52 patients, 43(82%) of them had thrombocytopenia. This prevalence is in accordance with the findings of Suma Rao[18].

6 patients had severe thrombocytopenia <20000/mm^3 and had bleeding tendency. Frank bleeding (Hematemesis) was noted in 2 cases. Bleeding during DHF may result from a combination of factors such as thrombocytopenia, coagulating defects and vasculopathy[19].

Table -4 shows Correlation of Dengue with organomegaly & thrombocytopenia. Hepatomegaly was noted in 15 (29%) patients and splenomegaly in 3 (7%) patients and Hepatosplenomegaly in 16 (31%) patients. This study coincided with the study conducted by S Chandy et.al which showed 42% incidence of hepatomegaly[3].

Table – 2 shows the clinical presentation of patients with dengue fever (seropositive).

Hanta virus infection can appear clinically uncharacteristic and may mimic other syndromes. This compounds the difficulties in diagnosing Hanta virus infection in areas where the disease is not endemic and clinical cases may be sporadic.[19].

In our study IgG/IgM Ab to Hanta virus was not detected.

The natural reservoirs of Hantavirus are small rodents and transmission to man is believed to occur via aerolized excretions. The worldwide distribution of rodents known to harbor Hanta virus suggest great disease causing potential[20]. The Thottapalayam virus, which belongs to the same family, was first isolated from spleen of a shrew capture in July 1964, in Vellore, India[21]. Very few reports from India are available in this regard. The vicinity of Mysore to Vellore may create an ecological predisposition for Hanta virus infection. In this regard an attempt was made to study the serological evidence of hanta virus infection, by detecting anti-hanta virus IgG/IgM antibodies.
To conclude, ICT helps in rapid diagnosis of Dengue and Hanta virus infection and also reduces the mortality & morbidity by starting early treatment. Hematological parameters like low platelet count is associated with complications of Dengue fever.

Table I: **Demographic profile**
Total no. of patients tested for Dengue serology: 1782
No. of patients with positive Dengue serology: 79(4.4%)
No. of cases reviewed (n): 52

Age in years	*Number(n=52)*	*Percentage%*
0 – 20	*18*	*35%*
21 – 40	*20*	*39%*
41 – 60	*9*	*18%*
>60	*3*	*06%*

Table II: **Presenting complaint on admission**

No. of cases reviewed (n): 52

Symptoms	*Number(n=52)*	*Percentage%*
Fever	*50*	*96%*
Vomiting	*18*	*34.6%*
Headache	*16*	*31%*
Myalgia	*11*	*21%*
Joint pain with swelling	*06*	*11%*
Loose stools	*08*	*15%*
Pain abdomen	*09*	*17%*
Cough with expectoration	*10*	*19%*
Rash	*01*	*02%*

Table III: Dengue seropositive with thrombocytopenia. (52 cases reviewed)

Dengue Serology	Thrombocytopenic(43)		Non- Thrombocytopenic(09)	
	Number	%	Number	%
NS1 Antigen	17	39	03	33
IgG	11	26	02	22
IgM	02	04	03	33
IgG+IgM	10	23	01	11
NS1+IgG	01	02	-	-
NS1+IgM	01	02	-	-
NS1+IgG+IgM	01	02	-	-

Table IV: Correlation of Dengue with Organomegaly & Thrombocytopenia

	Thrombocytopenic(43)		*Non- Thrombocytopenic(09)*	
	Number	*%*	*Number*	*%*
Hepatosplenomegaly	*14*	*33*	*02*	*22*
Hepatomegaly	*13*	*30*	*02*	*22*
Splenomegaly	*03*	*07*	*-*	*-*
No organomegaly	*13*	*30*	*05*	*56*

REFERENCES:

[1] Sjolander KB, Elgh F, Kokko HK, Vapalahti O, Hagglund M, Palmcrantz V, et al. Evaluation of serological methods for diagnosis of Puumala hantavirus infection (nephropathia epidemica). J Clin Microbiol 1997; 35: 3264-8.

[2] Chandy S, Abraham P and Sridharan G 2008 Hantaviruses: an emerging public health threat in India? A review; J. Biosci. 33 495-504.

[3] S.Chandy, S.Mitra, N.Sathish, T.S.Vijayakumar, O.C.Abraham, M.V.Jesudason, P.Abraham, K.Yoshimatsu, J.Arikawa & G.Sridharan, A pilot study for serological evidence of hantavirus infection in human population in South India, Indian J Med Res 122, September 2005, pp 211-215

[4] Wichmann D, Slenczka W, Alter P. Boehm S, Fieldmann H. Hemorrhagic fever with renal syndrome: diagnostic problems with a known disease. J Clin Microbiology 2001; 39:3414-6

[5] Ahlm C, Juto P, Stegmayr B, Settergren B, Wadell G, Tarnvik A, et.al Prevalence of serum antibodies to hantavirus in Northern Sweden as measured by recombinant nucleocapsid proteins. Scand J Infect Dis 1997;29:349-54.

[6] Chaturvedi UC, Shrvastavy R, Dengue haemorrhagic fever: A global challenge. Indian J Med Microbiol 2004;22:5-6.

[7] Tsai TF. Flavivirus, Yellow fever, Dengue DH, JE.St.Louisencephalitis, Tick-borne encephalitis. Chapter 142. In: principles and practice of infectious diseases, 5th ed. Mandell, Bennett, Dolin, Chruchill Livingston: Pennsylvania, USA:2000. P.1716, Indian J Medical Microbiology Vol.24, No.4

[8] Lanciotti RS, Calisher CH, Gubler DJ, Chang GJ, Vorndam AV. Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. J Clin Microbiol 1992;30:545-51.

[9] Siqueira JB, Martelli CM, Coelho GE, Simplicio AC, Hatch DL.Dengue and dengue haemorrhagic fever Brazil, 1981-2002, Emerg Infet Dis 2005; 2: 48-53.

[10] Narayanam M, Aravind MA, Ambikapathy P, Prema R, Jeyapaul MP, Dengue fever: Clinical and laboratory parameters associated with complications. Dengue bull 2003; 27:108-15.

[11] World Health Organization. Dengue Hemorrhagic Fever: Diagnosis, Treatment, Prevention and Control, 2nd edition. Geneve, World Health Organization, 1997.

[12] World Health Organization. WHO report on global surveillance of Epidemic prone infectious disease.

[13] http://www/who.int/emcdocuments/surveillance/docs/whocdscsrisr2001.html.

[14] Manjith Narayanan, M.A.Aravind, N.Thilothammal, R.Prema, C.S.Rex Sargunam and Nalini Ramamurthy, Dengue fever epidemic in Chennai – A study of clinical profile and outcome, Indian Pediatrics 2002; 39:1027-1033.

[15] Nicol ST. Bunyaviruses. In: Knipe DM, Howley PM, editors. Field's virology. 4th ed. Philadelphia: Lippincott William & Wilkins; 2001 p 1603-33.

[16] World Health Organization. Dengue haemorrhagic fever. Diagnosis, treatment, prevention and control. 2nd ed chapter 2. World Health Organization: Geneve, Switzerland; 1997.

[17] A Chakravarti, R Kumaria, N Berry and VK Sharma; Serodiagnosis of Dengue Infection by Rapid Immunochromatography Test in a Hospital Setting in Delhi, India, 1999-2001: Dengue Bulletin-Vol 26, 2002:107-112.

[18] Andrea J.Cuzzubbo, David W.Vaughn, Ananda Nisalak, Tom Soloman, Siripen Kalayanarroj, John Aaskov, Nguyen Minh Dung, and Peter L.Devine; Comparison of PanBio Dengue Duo Enzyme Linked Immunosorbent Assay(ELISA) and MRL Dengue Fever Immunoglobin M Capture ELISA for Diagnosis of Dengue Virus Infections in Southeast Asia. Clin Diagn Lab Immunol. 1999 September,6(5): 705-712.

[19] Sumarao Poorwosudarmo. Dengue haemorrhagic fever(thesis), University of Indonesia Press. 1983.

[20] Natth B. Pathology of Dengue Haemorrhagic fever: In monograph on dengue and dengue haemorrhagic fever. SEARO Regional Publication No.22, New Delhi, 1993, 22: 72-79.

[21] Schmaljohn C, Hjelle B. Hantaviruses: a global disease problem. Emerg Infect Dis.1997:3:95-104

[22] Carey DE, Reuben R, Panicker KN, Shope RE, Myers Rm, Thottapalayam virus: a presumptive arbovirus isolated from a shrew in India. Indian J Med Res 1971;59: 1758-60.