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Research Article

SERODIAGNOSIS OF DENGUE INFECTIONS IN ACUTE FEBRILE PATIENTS WITH THROMBOCYTOPENIA ALONG WITH SEASONAL VARIATION

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ABSTRACT
Background: Arboviral infections are one of the most important public health problems all over the world including India. Dengue virus is responsible for a growing health problem in the tropical and sub tropical countries. The global incidence of dengue fever and DHF has increased dramatically in recent decades. In 2006, there was a dengue outbreak in India affecting 11,637 individuals with case fatality rate of 1.49%.
Aim:
1) To identify dengue infection among febrile patients with thrombocytopenia attending a tertiary care hospital.
2) Seasonal variation of Dengue infection.
Materials and Methods: Febrile patients with thrombocytopenia with platelet count less than 1,00,000/cmm of all age groups and both sexes admitted at Tertiary Care Hospital, Mysore during the study period of one year six months (Jan 2012 – June 2013) were the subjects for this study. 2-3ml of venous blood was collected aseptically from these patients for serological test, after obtaining written informed consent. The serum was tested for IgM antibodies against Dengue virus by Enzyme Linked Immuno-sorbent Assay.
Results: In the present study, a total number of 200 cases of fever with thrombocytopenia were tested. Out of 200 suspected cases, 96 cases (48%) were confirmed as serologically positive for dengue infection. 31(32.3%) patients with Dengue infection were in the age group of less than 10 years. Children followed by young adults formed a major affected group. The mean platelet count of 96 dengue positive cases was 59,729 cmm. 4 (4.2 %) cases had platelet count in the range of <20,000/cmm. In the present study, maximum number of dengue cases was detected during the monsoon and post monsoon period.
Conclusion: Dengue infection was detected in all the age groups but predominantly noted in children and young adults. Dengue infection was related to rainfall as more number of cases were detected during monsoon and post monsoon seasons. Community awareness, early diagnosis and management and vector control measures need to be strengthened, during peri-monsoon period, in order to curb the increasing number of dengue cases.

Keywords: *Dengue infection, Platelet count, Rainfall, ELISA*

INTRODUCTION
Dengue virus is responsible for a growing health problem in the tropical and sub tropical countries. The global incidence of dengue fever and DHF has increased dramatically in recent decades (Joshi 2008; WHO; 2009). In India, dengue was first isolated in 1946, and many epidemics have since been reported. In 2006 there was a dengue outbreak in India affecting 11,637 individuals with case fatality rate of 1.49% (Joshi 2008).
Dengue virus, a member of the genus *Flavivirus* with its four sero-types is now classified within the Flaviviridae family and transmitted mainly by *Aedes aegypti* mosquitoes (Smith *et al.*, 2009; Gubler *et al.*, 2007). Among the four sero-types, infection with any of them generally leads to a mild self limiting febrile illness i.e. dengue fever. Its typical symptoms include headache, a characteristic skin rash, joint

pain and body ache. A more severe form of the disease involving vascular and haemostatic abnormalities leads to dengue hemorrhagic fever (DHF) and dengue shock syndrome, which is responsible for a high mortality rate, especially in children (Smith *et al.,* 2009; Gubler *et al.,* 2007).

The Aedes aegypti plays the role of vector for dengue virus, which is the day biter, domestic and peridomestic in nature and breeds in the household containers. The vector density rises after the monsoon period (Rajajee, 1995). These could be the amplifying hosts or reservoirs. For these reasons, the epidemics of these dengue infections occur every year in India (Taraphdar *et al.,* 2012). Dengue fever caused explosive epidemics affecting thousands of people all over the world and particularly in developing countries like India (Oishi *et al.,* 2007). Viral infections can be associated with reduced platelets either by inhibiting platelet production or causing their lysis. Thrombocytopenia associated with fever helps to narrow the differential diagnosis and management. Clinically, it is difficult to detect the causative pathogen of fever with thrombocytopenia. Laboratory help is very vital in identifying the cause. Isolation of the causative agent, demonstration of specific antigen and detection of specific antibodies are the common diagnostic tests adopted for detecting the arboviral infections. The increasing significance of Arboviruses as human and animal pathogens emphasizes that their study remains important. Serological tests have been the backbone for laboratory diagnosis of arboviral infections. IgM capture EIA can be used for early diagnosis of disease Joshi 2008, Schoub and Venter, 2009; Annane *et al.,* 2009).

A well organized approach that is carried out with an awareness of causes of fever with thrombocytopenia can shorten the duration of investigation and bring out the diagnosis. Accurate diagnostic tests have a key role in patient management and the control of most infectious diseases. This study attempts to detect the dengue infections as a cause of fever with thrombocytopenia among the patients admitted to Tertiary Care Hospital, Mysore.

Objectives:

1. To identify dengue virus infection among febrile patients with thrombocytopenia.
2. To identify the seasonal variation of Dengue infection.

MATERIALS AND METHODS

- Study design: Prospective study
- Duration of study: 1 year 6 months

Total number of 200 febrile patients with thrombocytopenia of all age group and both sexes admitted at Tertiary Care Hospital, Mysore during the study period of one year six months (Jan 2012 – June 2013) were the subjects for this study. A written informed consent was obtained from all the patients who participated in the study after explaining the patient's diagnosis, the nature and purpose of the study. Institutional ethical committee clearance was obtained.

Sample Collection and Test Procedure:

2-3ml of venous blood sample was collected by venipuncture in a plain vacutainer from patients with standard precautions. Sample was allowed to clot at room temperature and then centrifuged. The serum was separated and tested for IgM antibodies against Dengue, using commercially procured In-Vitro Diagnostic (IVD) approved PanBio ELISA Kits.

Statistical Analysis: The data is analyzed using appropriate SPSS software version 20. Percentage analysis of the data will be given.

Case-inclusion criteria:

Febrile patients with thrombocytopenia with platelet counts less than 1,00,000/cmm of both sexes and all age groups.

Exclusion criteria:

1. Thrombocytopenic patients with no fever and patients with prolonged fever.
2. Other infectious causes like malaria, leptospirosis, enteric fever and scrub typhus.
3. Fever with localized focus of infection in skin, soft tissues, connective tissue, respiratory, gastrointestinal and genitourinary system and autoimmune diseases.

4. Idiopathic thrombocytopenic purpura, Leukaemia, Malignancy.

RESULTS

In the present study, a total number of 200 cases of fever with thrombocytopenia were included, for the period of 18 months from January 2012 to June 2013. Out of 200, 111 (55.5%) were males and 89(44.5%) were females. 58 (29%) patients were in the age group of less than 10 years and formed a major group presenting with febrile thrombocytopenia. In the present study, the mean platelet count of the patients with febrile thrombocytopenia was 62,022/cmm. (Table 1)

Among 200, 96(48%) of patients with febrile thrombocytopenia were positive for Dengue IgM ELISA. Among Dengue positive cases 49 (51%) were males and 47(49%) were females. In the present study, 31(32.3%) patients with Dengue infection were in the age group of less than 10 years. Children followed by young adults formed a major affected group. (Table 2). 4 (4.2 %) cases had platelet count in the range of <20,000/cmm, 19(19.8%) cases ranged from 21,000-40,000 /cmm, 21(22%) cases ranged from 41,000-60,000/cmm, 28(29%) cases ranged from 61,000-80,000/cmm and 24(25%) cases ranged from 81,000 - 1,00,000/cmm. (Chart 1) In the year 2012, number of dengue cases were increased from the month of June and reached peak in October. In the year 2013, number of dengue cases were increased from the month of May. (Chart 2) According to intensity of rainfall, weather data has been divided in three periods, namely; pre monsoon period from February-May, monsoon period from June-September and post monsoon period from October-January. In the present study, maximum number of dengue cases were detected during the monsoon and post monsoon period.

The difference between numbers of serologically positive cases during different months was significant. Larger proportions of serologically positive cases were observed among children. Outbreak coincided mainly with the post monsoon period of subnormal rainfall. The difference between serologically positive cases as compared to serologically negative ones in post monsoon period was significantly higher. The difference in the rainfall and temperature between three seasonal periods was significant.

Table 1: Distribution of platelet counts in Fever with Thrombocytopenia cases

Platelet Count/cmm	Number of Patients	Percentage (%)
0-20,000	9	4.5
21,000- 40,000	30	15
41,000- 60,000	53	26.5
61,000-80,000	52	26
81,000-1,00,000	56	28
Total (n)	200	100

Table 2: Age distribution among Dengue positive cases

Age (years)	Number of cases	Percentage (%)
0-10	31	32.3
11-20	20	20.8
21-30	23	24
31-40	11	11.4
41-50	04	4.2
51-60	05	5.2
>61	02	2.1
Total Dengue cases	96	100

Chart 1: Platelet Counts distribution among Dengue positive cases

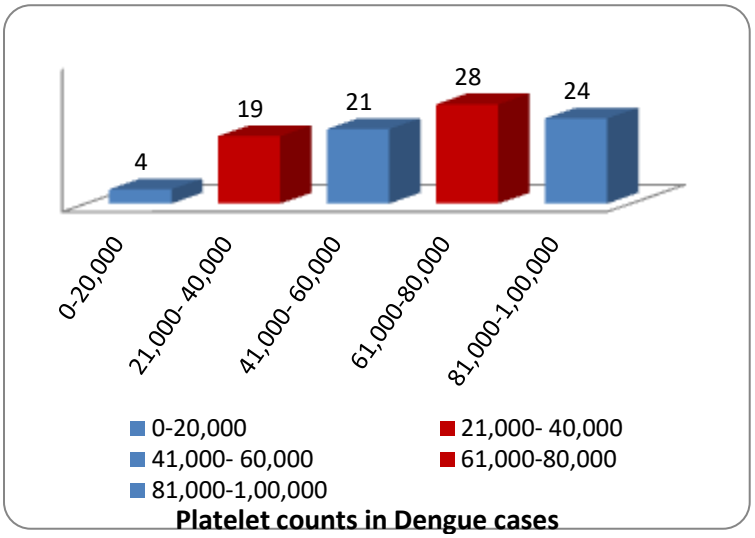
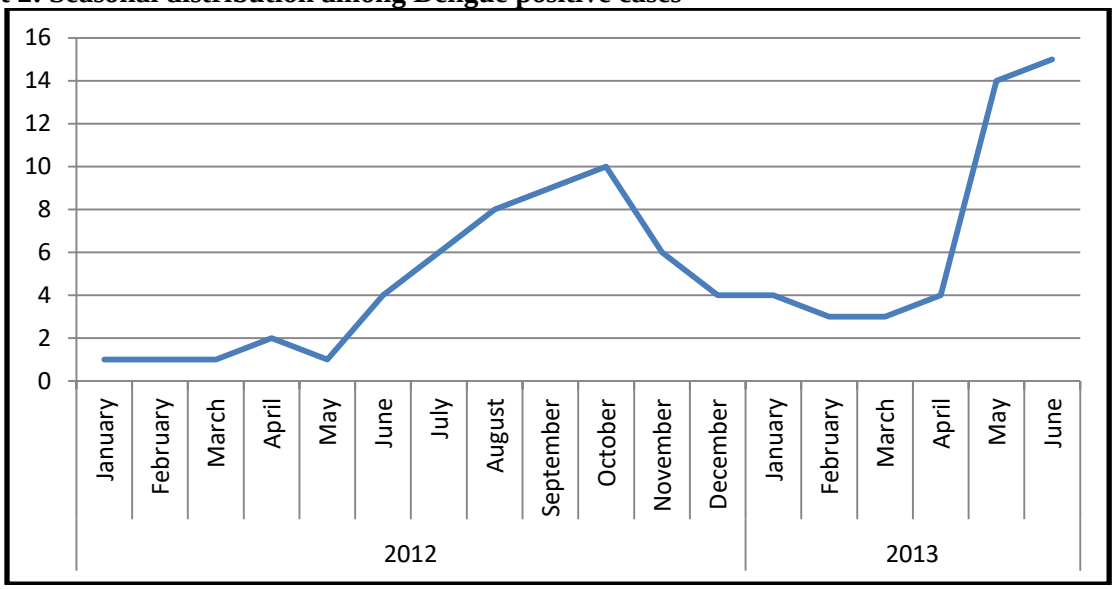


Chart 2: Seasonal distribution among Dengue positive cases



DISCUSSION

Arboviruses are major causes of fever in endemic areas of the world. Many a times infections by these viruses are subclinical or mild, occasionally may cause severe hemorrhagic illness. Laboratory diagnosis by isolation of virus, by detection of viral genome or by demonstration of a rise in antibody is possible in special centers (Mims *et al.,* 2004). Tests for viral antigens and antibodies are now based on ELISA technology; often with use of recombinant antigens and monoclonal antibodies as sources of defined reagents (Monath and Tsai 2002). Serological tests have been the backbone for laboratory diagnosis of arboviral infections. IgM capture EIA can be used for early diagnosis of disease (Chairulfatah *et al.,* 2003).

Dengue disease was first described in the 18th century, but the causative agent was isolated in 1944. Between 1944 and 1956 it was shown that four distinct viruses, designated dengue virus types 1 to 4, were responsible for the same clinical syndrome.[10] In the period 1980-2006 there has been widespread occurrence of DHF in different parts of India. On a global scale, dengue is now the most common and clinically serious VHF, even though only a small fraction of secondary infections lead to the shock syndrome.

The present, serological based study was done to detect the presence of dengue infections in 200 patients of acute febrile illness with thrombocytopenia after excluding the cases which were positive for Malaria, Leptospira, Scrub typhus, Enteric fever and infections with localized and systemic manifestations by doing appropriate laboratory investigations.

In the present study, out of 200 cases of acute fever with thrombocytopenia, 111 (55.5%) were males and 89(44.5%) were females. Patients of all age group were included and ranged between 6months – 75 years. But 71% were in the age group of 0-30yrs. 131(65.5%) cases had platelet count in the range of 50,000 - 1,00,000 /cmm followed by 60(30%) who had a platelet count in the range of 20,000-50,000 /cmm and 9(4.5%) had a platelet count below 20,000 /cmm.

In the present study, 96(48%) cases were serologically positive for Dengue infection with 95% Confidence Interval which varies from 40.93 – 55.06.

In a study conducted by Deol Siddhart *et al.,* (2003) on 33 patients of viral haemorrhagic fever between June 2002 to May 2003 in Miraj, 31 were serologically positive for dengue infection and formed a predominant cause. Similar to, Deol Siddhart *et al.,* (2003) dengue infection was common in the present study as well.

In the present study, Dengue positive cases were almost equally distributed among both the genders, with 49 (51%) male and 47(49%) female cases. In the study conducted by Alex (Chairulfatah *et al.,* 2003). among 1300 DHF/DSS cases, 52% were male and 48% were female. The present study results on gender distribution of Dengue infection are comparable with that of Alex Chairulfatah *et al.,* (2003) study with equal sex distribution.

In the present study, 31(32.3%) patients with Dengue infection were in the age group of less than 10 years. Children and young adults formed a major affected group (77%). 32.3%, 20.8% and 24% cases were in 1st, 2nd and 3rd decade respectively. Alex Chairulfatah *et al.,* (2003) found 38%, 31% and 18% cases in 1st, 2nd and 3rd decade respectively (Chairulfatah *et al.,* 2003). Deepti Pruthvi *et al.,* (2012) found that, 65.9% of serologically positive dengue cases belonged to pediatric (<12 years) age group in Davanagere in the year 2009.

Age distribution of dengue virus infection in the present study was similar to that observed in the above two studies of Alex Chairulfatah *et al.,* (2003) and Deepti Pruthvi *et al.,* (2012) indicating the children and young adults as the vulnerable group.

In the present study, among 96 dengue positive cases, 4 (4.2 %) cases had platelet count in the range of <20,000/cmm, 19(19.8%) cases ranged from 21,000-40,000 /cmm, 21(22%) cases ranged from 41,000-60,000/cmm, 28(29%) cases ranged from 61,000-80,000/cmm and 24(25%) cases ranged from 81,000 - 1,00,000/cmm. Among 4 dengue infected case with platelet count <20,000/cmm, two patients improved with platelet transfusion and two patients improved with Intravenous (IV) fluids.

Seasonal variation in dengue is noticed worldwide and it is related to rainfall. Worldwide studies have proposed that ecological and climatic factors influence the seasonal prevalence of both the *Aedes aegypti* and dengue virus. This can be attributed to the stagnation of water after rainfall which facilitates vector breeding (Deepti Pruthvi *et al.,* 2012). In the present study during the year 2012, number of dengue cases were increased from the month of June and reached peak in October. In the year 2013, number of dengue cases increased from the month of May. Thus, maximum numbers of dengue cases were seen during monsoon and post monsoon period.

In the study done by Deepti Pruthvi *et al.,* (2012), authors observed 159(54.1%) cases in the monsoon period, 107(36.4%) cases in the post monsoon and 28(9.5%) cases in the pre-monsoon period were

serologically positive for Dengue virus infection. According to author the temperature tends to decrease towards the end of monsoon period and remains constant during the later months of rainy season. India falls in the deciduous, dry and wet climatic zone. The temperature remains high during the pre monsoon period. It is continuous rain pour for a couple of days that brings down the temperature during the monsoon period, which may also be responsible for an increase in the relative humidity and decrease in the evaporation rate thus maintaining secondary reservoirs containing rain water (Deepti Pruthvi *et al.,* (2012).

Ashwini kumar *et al.,* (2010) also observed increased number of Dengue cases in the monsoon and post monsoon season in Manipal, Karnataka. The correlation between the occurrence of dengue and the monsoon season was evident and is further supported by similar findings in Kerala (Kavitha *et al.,* 20070, Ludhiana (Punjab) (Madn Lal et al., 20070; and Karachi (Pakistan) (Khan *et al.,* 2007).

Recommendations for future research: The re-emergence and persistence of dengue virus suggests the need for continuous monitoring and identification of the newly evolving variants and their genetic divergence with a view to plan for appropriate strategies for containment of dengue infections and vaccine development as well.

Further studies on the vector prevalence, the environmental conditions that favor the persistence of dengue infections, serosurveys in humans and mosquitoes in the affected areas are required to better understand the dynamics of dengue infections and prevent outbreak.

CONCLUSION

This study highlighted rain, temperature and relative humidity as the major and important climatic factors, which could alone or collectively be responsible for an outbreak, and also the drastic fall in the platelet count which is life threatening is highlighted. More studies in this regard could further reveal the correlation between the climatic changes and dengue outbreaks which would help in making the strategies and plans to forecast any outbreak in future well in advance.

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