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Evaluation of Platelet Count in Dengue Fever Along with Seasonal Variation of Dengue Infection

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Abstract

Introduction: Indian Subcontinent has emerged as a scene for mosquito-borne infectious diseases, like Malaria and Dengue fever. After the 1990s, the rate of Malaria has declined owing largely to preventive measures, but at the same time the rate of Dengue Fever (DF) and dengue hemorrhagic fever has increased to a larger extent.

Objectives: 1) Evaluation of platelet count and the prevalence of Dengue Fever. 2) Seasonal variation of Dengue infection

Materials and methods: The present study was conducted retrospectively for a period of 1 year during the recent outbreak of dengue fever in Davangere in the year 2009. Blood samples were collected from 1549 patients experiencing a febrile illness, clinically consistent with Dengue infection. Serological confirmation of Dengue Infection was done and platelet count was done in all the serologically positive cases.

Case-inclusion criteria: All patients with clinical features and serologically positive dengue infections were included.

Exclusion criteria: 1) Patients with thrombocytopenia but serologically negative were not included. 2) Patients with thrombocytopenia and no fever were not included. 3) A case was excluded, if routine laboratory testing suggested bacterial or any viral infection other than dengue infection or any other disease.

Results: Out of 1549 suspected cases, 294 cases (18.97%) were confirmed as serologically positive. The difference between numbers of serologically positive cases during different months was significant. Larger proportions of serologically positive cases were observed among adults. 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.

Conclusion: This retrospective 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, platelet count and dengue outbreaks, which would help in making the strategies and plans to forecast any outbreak in future well in advance.

Keywords: Dengue infection; Platelet count; Dengue fever; India; Rainfall; Temperature; Relative humidity

Introduction

The Indian Subcontinent has emerged as a scene of many mosquito-borne infectious diseases, including malaria and dengue fever. After the 1990s, the rate of malaria declined owing largely to preventive measures, but at the same time Dengue Fever (DF) and Dengue Hemorrhagic Fever (DHF) were increasing in the region.

Dengue is the most prevalent mosquito-borne viral infection worldwide, with 100 million cases of Dengue Fever (DF) and half a million cases of Dengue Hemorrhagic Fever (DHF) annually [1,2]. Since 1989, there have been regular epidemics of DHF in India. During the past few years, the characteristics of dengue in India appear to have changed. For instance, a decade ago, children were predominantly affected, but in recent years clinicians have seen increasing numbers of adult dengue patients, with both significant morbidity and increasing numbers of adult deaths due to dengue.

Similar recent trends of increasing numbers of adult dengue patients can also be seen in other South Asian, South-East Asian and Latin American countries [2-7]. If we are to take effective steps to reduce this trend and treat this group optimally, pooling information from different countries is important. At present, information on adult dengue infections in South Asia is quite limited. We have seen an increase in dengue infections among adults in and around Davangere with similar trends seen in many parts of our country. Data on the natural history and outcome of dengue in adults is quite limited.

Dengue fever is an acute febrile illness caused by four serotypes of Dengue virus and in older children characterized a spectrum of disease, biphasic fever, myalgia, arthralgia, rash and leucopenia. Dengue Hemorrhagic Fever (DHF) is characterized by hemoconcentration, abnormality of hemostasis and in severe cases by a fluid & protein losing shock syndrome (Dengue Shock Syndrome, DSS).

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This arthropod born virus is transmitted by a day time biting mosquito *Aedes aegypti*. There is no cross protection between the 4 dengue serotype but there is cross reaction [1]. The disease usually establishes a pattern of epidemic activity every 2-5 years.

According to WHO criteria [3], for defining DHF the following must all be present (a) fever, (b) hemorrhagic tendency (c) thrombocytopenia (d) evidence of plasma leakage, manifested by either a rise in the hematocrit equal to or greater than 20% above average for age, sex and population or signs of plasma leakage such as pleural effusion, ascites and hypoprotienemia. For defining DSS all of the above four criteria for DHF plus evidence of circulatory failure manifested by rapid and weak pulse, narrow pulse pressure (less than 20 mmHg), hypotension for age or cold, clammy skin must be present.

DHF/DSS occurs with higher frequency in two immunologically defined groups: children who have experienced a previous dengue infection, and infants with waning levels of maternal dengue antibody [8].

In majority of patients thrombocytopenia is transient and asymptomatic but in significant number of cases there is bleeding manifestations [9]. Spontaneous bleeding is noted in platelet count of <20,000 in majority of patients. Petichae/purpura is seen in platelet count in the range of 20,000-40,000. This signifies the need to evaluate platelet count and the prevalence of Dengue Fever with thrombocytopenia and the follow up after platelet transfusion.

Dengue has emerged as a disease in adults and in pediatric patients with a year round incidence in and around Davangere. The clinical features of 1549 cases with laboratory proven Dengue infection who were hospitalized in SS Institute of Medical Sciences and Research Center, Davangere from-January 2009 to December 2009 were described. We discuss annual cases of DF/DHF in Davangere and possible factors involved in DF outbreaks and also climate as an important factor influencing DF outbreaks, and rainfall, temperature and humidity play a pivotal role in DF outbreaks. Clinicians must be well versed with the clinical presentations, the initial diagnosis, and the platelet count and has to evaluate it thoroughly.

Objectives

1. Evaluation of platelet count and the prevalence of Dengue Fever.
2. Seasonal variation of Dengue infection.

Materials and Methods

Study design, population and sample size

The present study was conducted retrospectively for a period of 2 years during the recent outbreak of dengue fever in Davangere in the year 2009. Blood samples were collected from 1549 patients experiencing a febrile illness clinically consistent with dengue infection. Serological confirmation of Dengue Infection was done.

Source of data

This study consisted of 1549 patients with history of fever with thrombocytopenia, subjected to serological tests, and patients serologically confirmed as dengue infections from both inpatients and outpatient departments at SS Institute of Medical Sciences and Research centre, Davangere during the period January 2009 to December 2009.

Method of collection of data

Clinical and laboratory information was collected from 1549 patients amongst whom 264 were serologically confirmed as dengue infections treated in SS Institute of Medical Sciences and Research centre, Davangere. Platelet count was repeated regularly during the hospital stay and also at the time of discharge hematological laboratory measurements was recorded serially until discharge from hospital.

Case-inclusion criteria

All patients with clinical features and serologically positive dengue infections were included.

Exclusion criteria

1. Patients with thrombocytopenia but serologically negative were not included.
2. Patients with thrombocytopenia and no fever were not included.
3. A case was excluded, if routine laboratory testing suggested bacterial or any viral infection other than dengue infection or any other disease.

Laboratory confirmation of dengue infection by serology

Dengue Duo IgM and IgG Rapid Strip test (Pan Bio, Australia) was used for the detection of dengue-specific antibodies. The presence of anti-dengue IgM antibodies alone indicated primary infection. In contrast, presence of anti-dengue IgG antibodies with or without IgM

Year 2009	Total no of suspected cases of dengue	Total no of suspected pediatric cases	Total no of serologically positive pediatric cases	Total no of suspected adult cases	Total no of serologically positive adult cases	Total no of Serologically positive cases
January	37	23	11	14	4	15
February	17	9	2	8	1	3
March	28	14	1	14	3	4
April	31	18	4	13	3	7
May	52	20	11	32	3	14
June	107	65	27	42	4	31
July	186	94	35	92	5	40
August	277	124	33	153	12	45
September	248	130	29	118	14	43
October	278	148	21	130	25	46
November	158	98	18	60	05	23
December	130	88	12	42	11	23
Total	1549	831	174	718	90	264

Table1: Month wise distribution of clinically diagnosed and serologically positive cases amongst primary and secondary cases during the DF outbreak, January 2009-December 2009.

Platelet count	<25,000	25,000-49,000	50,000-74,0000	75,000-1,00,000	1,00,0000
No of cases	472	386	376	169	146

Table 2: Platelet count.

indicated secondary infection. (IgG antibodies alone was considered as suspected secondary infection as it could also be due to cross reactivity with other flaviviruses) (Table 1,2).

Results

Out of 1549 suspected cases, 264 cases (17.04 %) were confirmed as serologically positive. The difference between numbers of serologically positive cases during different months was significant. Larger proportions of serologically positive cases were observed among adults. 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**.**

Evaluation of platelet count

Platelet count was done in all the serologically positive cases out of which 472 cases (30.47%) the platelet count was <25,000. 386 cases (24.91%) ranged from 25,000-49,000. 376 cases (24.27%) ranged from 50,000-74,000. 169 cases (10.91%) ranged from 75,000-1,00,000. 146 cases (9.42%) were above 1,00,000.

Laboratory analysis

Laboratory diagnosis of dengue virus infection can be made by detection of the specific virus, viral antigen, genomic sequence and their antibodies. Here in this study dengue was confirmed in 264 cases out of 1549 patients by IgM ELISA Analytical interpretations presented in this study were based upon instructions mentioned in the Pan Bio Rapid Strip Test procedure manual. During the outbreak period, blood samples were collected from 1549 patients experiencing a febrile illness clinically consistent with DI over the period of one year from January 2009 to December 2009. 264 cases (17.04%) were confirmed as serologically positive. The difference between numbers of serologically positive cases reported during different months was significant.

Seasonal variation

Dengue Infection is observed to be a seasonal disease. According to intensity of rainfall, weather data was divided in three periods, namely; pre monsoon period: from February-May, monsoon period: from June-September and post monsoon period: from October-January. Few cases clinically suspected of dengue infection in the pre monsoon period were later found to be serologically negative for dengue-specific antibodies. During the monsoon period, only 159 cases (60.2%), post monsoon 107 cases (40.5%) and pre-monsoon period 28 cases (10.6%) were confirmed serologically positive. The difference between numbers of serologically positive cases as compared to serologically negative ones in post monsoon period was significantly higher.

Distribution by age

Out of 264 serologically positive cases 90 (34.09%) cases belonged to the adult's age group (>12 years) and 174 (65.9%) cases to paediatric age group (≤ 12 years) in this study. Larger proportions of serologically positive cases were observed among paediatric cases. The difference between numbers of serologically positive cases among adult and paediatric group in post monsoon period as compared to the rest of the season was also not significant.

Climatic influence and the relationship to relative humidity and rainfall

This study indicates that outbreak coincided mainly with the post monsoon period of subnormal rainfall and was followed by relatively heavy rainfall during the monsoon period; from June to September 2009. The difference in the rainfall and temperature between three seasonal periods was found to be significant. Mean ambient temperature was 25.4°C during the pre-monsoon period, which increased to 30.9°C during the monsoon period; the period preceding the outbreak and decreased to 20.3°C (Mean temperature from October to December) in the actual outbreak months during the post monsoon period.

Discussion

In the year 2009 India had experienced one of the wettest monsoons in 25 years, which led to a spate of mosquito growth creating an alarming situation of mosquito borne diseases in Davangere and many other states. As a consequence to this unusually heavy rain, an outbreak of dengue fever was once again reported from Davangere after a silence of six long years. Most of vector borne diseases exhibit a distinctive seasonal pattern and climatic factors such as rainfall, temperature and other weather variables affect in many ways both the vector and the pathogen they transmit. Worldwide studies have proposed that ecological and climatic factors influence the seasonal prevalence of both the *A. aegypti* and dengue virus. The vector mainly responsible for the spread of DI is present at the basal level all the year around in Davangere; however, studies on the relative prevalence and distribution have shown the highest *A. aegypti* larval indices during the monsoon and post monsoon period. Since limited data is available on the affect of climatic factors on the pattern of DI, this study was planned to carry out the month wise detailed analysis of three important climatic factors such as rainfall, temperature and relative humidity on the pattern of DI.

Observations on the seasonality were based on a single year's data as the intensity of sampling was at its maximum during this outbreak period. The outbreak coincided mainly with the post monsoon period of subnormal rainfall, which was followed, by relatively heavy rainfall during the monsoon period; from June to September 2009. The difference in the total rainfall and temperature during three seasonal periods was found to be statistically significant. Even though, the monsoon season began in mid-June, there was no respite from the heat as there was not much difference in the temperature during the last month of pre monsoon May and beginning of monsoon in the June. Unusual heavy rainfall subsequently led to decrease in temperature during the later part of monsoon period. The temperature showed a decline and remained almost constant during the months of July and August (30.2°C), continuous heavy rainfall subsequently led to further decrease in the temperature during the month of September to 29°C. Relative humidity increased during the rainy season and remained high for several weeks. An in-depth analysis of these three factors thus led to a proposal that optimum temperature with high relative humidity and abundant stocks of fresh water reservoirs generated due to rain developed optimum conditions conducive for mass breeding and propagation of vector and transmission of the virus.

There was a significant drop in the platelet count which is life threatening, 78% of our case had platelet count below 75,000 which seeks for the need of platelet transfusion and single donor platelet transfusion thus reducing the refractoriness of the platelet transfused.

Our study was in tune with seasonal variations and breeding pattern of *A. aegypti* in Davangere, which showed that there are two types of

breeding foci, namely; primary and secondary breeding foci. Primary breeding foci serves as mother foci during the pre monsoon period. *A. aegypti* larvae spreads to secondary foci like discarded tyres, desert coolers etc., which collect fresh water during the monsoon period. This study supported the proposal that all the three climatic factors studied could be playing an important role in creating the conducive condition required for breeding and propagation of this vector, the basal level of which is present all round the year. This study therefore highlighted the major important factors, which could alone or collectively be responsible for an outbreak.

In our study, the largest proportion of serologically positive cases was recorded in the post monsoon period, which is in agreement with our previous study. Our findings were in coordination with study by other groups from this geographical region. The seasonal occurrence of positive cases has shown that post monsoon period is the most affected period in Bangladesh as well [10]. However, a retrospective study from Myanmar during 1996-2001 reported the maximum cases of dengue during the monsoon period Chakravarti and Kumaria [11] and the Study by group of Rebelo from Brazil has also emphasized the importance of season. They have observed that dengue cases were higher during rainy season showing the importance of rain in forming prime breeding sites for *A. aegypti* thus spread of DI [11]. Study of eco-epidemiological factors by Chakravarti and Kumaria [11] showed that DF has a positive correlation with the relative humidity and negative relation with evaporation rate. Peaks of dengue cases were observed to be near concurrent with rain peaks in this study from Venezuela showing a significant correlation of intensity of DI with the amount of rain. In this study we have observed that temperature tends to decrease towards the end of monsoon period, specially remains moreover constant during the later months of rainy season. India and Bangladesh fall 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. More studies are needed to establish the relationship between the climatic changes and dengue outbreaks, which would help in formulating the strategies and plans to forecast any outbreak in future, well in advance.

Dengue is found rarely in adults in Thailand, presumably because people acquire complete protective immunity after multiple DI as children, as DI is highly endemic in Thailand [11]. On the other hand, DI especially DHF is an emerging disease in India; probably this may be the reason that people of all the age are found to be sensitive to infection in our study. Even though more adults were reported of having anti dengue antibodies, the difference in the number of positive cases was not significant as compared to paediatric age group.

Conclusion

This retrospective 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. Platelet count 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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