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CLINICAL AND VIROLOGICAL STUDY OF DENGUE : AN INSTITUTIONAL EXPERIENCE

KEYWORDS: Dengue Fever, Denhue haemorrhagic fever, Serotype

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ABSTRACT *Aims and Objectives: This study was designed to investigate the prevalence and clinicopathological profile of Dengue in JA group of hospitals.*

Material & Method: Serum samples were obtained from IgM positive dengue patients referred to the various departments during May 2015 to April 2016, and detailed history was taken on a pre-structured datasheet. All samples were tested for hematological and biochemical profile. NS1Ag positive samples were tested further by conventional RT-PCR for DV-RNA detection and serotyping.

Result: Prevelance of dengue was 24.44%, with DF: DHF ratio of 3.34:1. The most affected age group was 11-20 years followed by 21-30 yers. Males are more affected than females. Maximum cases were reported in month of October. DENV -2 strain was the most common strain found in this region.

Conclusion: Dengue is common cause of illness in febrile patients coincide with post monsoon period. The study serves as the baseline data about circulation of dengue viruses in Gwalior region. Regular continuous surveillance is warranted to monitor the circulation of dengue virus in future.

INTRODUCTION

Dengue virus (DENV) infection is one of the mosquito-borne viral diseases with a major impact on public health, globally [1]. About 3.5 billion people, 55% of the world's population living in tropical and subtropical regions is at risk, with about 50 million DENV infections occurring annually and approximately 500,000 requiring hospitalization annually [2]. The average case fatality rate is around 5%, and mainly among children and young adults[3].

Dengue is a mosquito-borne viral illness caused by one of the four serotypes of the dengue virus DENV (DENV-1 to DENV-4) belonging to the family Flaviviridae. They are transmitted mainly by the Aedes aegypti mosquito and also by Aedes albopictus [4]. Dengue has been an urban disease but now has spread to rural areas of India as well [5].

Dengue virus infection is a complex disease with symptoms being difficult to distinguish from other common febrile illnesses during acute phase and can progress from a mild, non-specific viral disease to severe cases characterized by thrombocytopenia, hemorrhage manifestations and hemo-concentration due to plasma leakage.

Majority of febrile illnesses in Gwalior district are treated as presumptive malaria, often without proper medical examination and a laboratory diagnosis. Therefore, many patients with fever are designated as having fever of unknown origin or malaria and remain without a laboratory diagnosis even if they fail to respond to antimalarial drugs. The scenario indicates that many cases of DENV infections are undiagnosed or even misdiagnosed. Despite the all efforts true prevalence of Dengue is not known in Gwalior region hence this study was planned to evaluate the demography and clinic-pathological profile of dengue fever in Jayarogya Group of Hospitals (JAH).

MATERIAL AND METHODS

Study design: This study was a hospital-based prospective study conducted for a period of 1 year (May 2015 to April 2016) in Department of Pathology, Gajra Raja Medical college (GRMC) and associated Jayarogya group of hospital (JAH) Gwalior in association with Defence Research and Development Establishment (DRDE) Gwalior. The institutional ethical committee approved the study protocol.

Participants: The study group consists of 100 patients admitted in our hospital with dengue Immunoglobin M (IgM) positive. Patient suffering from any infection, viral hepatitis during this period were excluded from study.

Case Definition[6]:

Dengue fever: Acute onset of high fever 3–14 days after the bite of an infected mosquito. Symptoms include frontal headache, retro-orbital pain, myalgias, arthralgias, rash, and low white blood cell count.

Dengue haemorrhagic fever: World Health Organization (WHO) criteria:

1. Fever or recent history of fever lasting 2–7 days.
2. Any hemorrhagic manifestation.
3. Thrombocytopenia: Platelet counts less than 100×10^9/L
4. Evidence of increased vascular permeability.

Dengue Shock Syndrome: defined as any case that meets the four criteria for DHF and has evidence of circulatory failure manifested by (1) rapid, weak pulse and narrow pulse pressure (≤20 mmHg [2.7 kPa]) or (2) hypotension for age, restlessness, and cold, clammy skin.

Method: Demographic and clinical characteristics of the participants were recorded on a self-designed semi structured per-forma in all these patients. Just after admission, 5ml. of blood was collected aseptically from each patient. 1 ml of clotted blood was used for Immunoglobin M (IgM) antibody by mac ELISA; remaining 2 ml of collected blood sample was kept in EDTA vial and was used for haematological investigations by manually as well as by 5 part analyser. Rest 3 ml of clotted blood

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after separating serum was used for biochemical test and NS1 Antigen. Serum of NS1 Antigen positive patients were further subjected for RT PCR & Real Time PCR using the QIAamp viral RNA mini kit (Qiagen, Germany) and ABI 7500 fast Dx Real time PCR instrument using the dengue specific primer and probes by CDC, USA for virus serotype isolation.

Statistical analyses

Data were analyzed using SPSS version 20, with a 2-tailed α level of 5%. Statistical analyses were performed by Chi-square tests analyses. The criterion for statistical significance was set at p_0.05, and for statistical trend at p_0.10. Given the small sample size, our hypothesis testing must be considered preliminary rather than definitive.

RESULTS

Epidemiological profile of DENV IgM Positve patients: Total of 409 cases of clinical fever was admitted in various departments during May 2015 to April 2016 in JA group of Hospitals. 100 cases were positive for IgM, prevalence of dengue seropositive was 24.44%. 80 percent were males (n=80) and 20 percent (n=20) were female. Male: Female ratio was 4:1. Dengue fever was present in 77% while 23% have dengue haemorrhagic fever. We did not found any case of DSS. The age of all patients ranged from 6 month to 69 years. The mean age was 16 years (SD 10.69) and 24.09 years (SD 12.38) for DHF and DF respectively. Figure1 shows the monthly distribution of reported DENV infection cases; maximum number of dengue fever commenced during October and September followed by November and least in the month of March, February, and May.

Table : Epideomiological profile of DENV IgM Positve patients

Variable	N	Dengue Fever	Dengue Haemorrhagic Fever	X^2 test
Dengue severity	100	77	23	
Sex				p <. 722,df 1
Male	80	61(79.22%)	19(82.60%)	
Female	20	16(20.77%)	4(17.39%)	
Age (Years)				p <. 331 df 6
0-10	21(21%)	12(15.58%)	9(39.13%)	
0-1	2(2)	2(2.59%)	0(0%)	
1-10	19(19%)	10 (12.98%)	9(39.13%)	
11-20	29(29%)	23(29.87%)	6 (26.08%)	
21-30	27(27%)	22(28.57%)	5(21.73%)	
31-40	17(17%)	15(19.48%)	2(8.69%)	
41-50	4(4%)	3(3.89%)	1(4.34%)	
51-60	1(1%)	1 (1.29%)	0	
61-70	1(1%)	1(1.29%)	0	

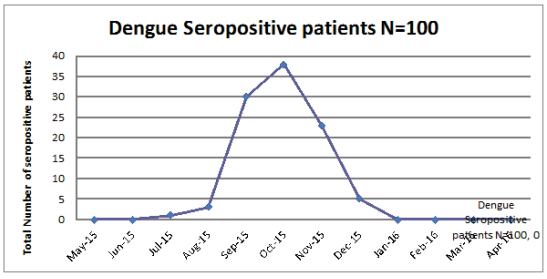

Figure 1: Marked Line Diagram Showing Seasonal Variation of DENV Infection

DENV Serotype: 35 samples were found positive for NS1 Antigen (Table 2). A total of 8 samples (22.85%)found to be positive for RT-PCR &Real Time PCR (Table 3). DENV 2 was the most common strain found, followed by coinfection of DENV 2,3 & DENV 2,3,4 . DENV 1 was found only in one case (Table 2).

Table 2: Serological Profile of Dengue Seropositive Patients

Test	N	%
IgM	65	65
NS1+ IgM	35	35
Total	100	100

Table 3 : Distribution of cases showing RT-PCR positive results according to age and sex

Patient ID	Age (years)	Sex	DENV strain
Sample 1	9	M	DENV 2
Sample 2	38	F	DENV 2
Sample 3	23	M	DENV 2
Sample 4	22	M	DENV 2
Sample 5	7	M	DENV 1
Sample 6	29	M	DENV 2
Sample 7	15	M	DENV 2 + 3
Sample 8	13	M	DENV 2 + 3 +4

DISCUSSION

Either one or more of the two serological markers (NS1, IgM) were positive in 24.44% cases tested. Various studies have reported a seropositivity ranging from 15.2% (Kulkarni RD et al, 2011)[7] to 39.41% (SantoshTathe et al, 2013) [8] for one or more serological markers of dengue. The present study findings appeared to be lower as compared to 31.3% reported by PM Ukey et al. [9] and higher than 22% reported by Om prakash et al 2015[10]. The present findings may be as due to the spatial diffusion of the virus and vector proliferation within the Gwalior region.

Our study revealed the most susceptible age group for dengue fever was 11-20 years and followed by 21-30 years suggesting that the individuals in these age groups were actively involved in outdoor activities that increased their chances of exposure to the infective DENV vector bite, however DHF were more in 0-10yrs of age in compare to higher age groups, probable explanation for these finding were passive transfer of antibody from the mother or previous infection. Study done by Narayanan et al [11] revealed DF was more common in 7 to 8 years of age, which was similar to the study done by Kabra SK[12] et al and Banik GB[13] et al. Regarding children, a lower DENV infection was observed in age group < 1 year (2%) in respect to 1-10 years (19%) in our study. Since the vector Ae.aegypti, is a predominantly day biting outdoor vector, Children < 1 year were at a lower risk of dengue infection as they spend most of their time indoors, completely covered or sleep under bed nets unlike the children aged 1-10 year who were able to play and spend more time outdoors within and around the residential areas.

Few available hospital studies demonstrated male-female distributions in dengue fever. Kabra [12]SK et al showed girl preponderance as also seen in the study done by Mittal H[14] et al. Three independent studies in India and Singapore showed that males were twice more common than girls. Hospital based study in Delhi showed male to female ratio 2.5:1. [15].Similarly in our study, there were more involvement of male compare to females (4:1), more probable explanation of these finding were under reporting of female to health care system in India as well as more outdoor activity by male compare to females.

In the present study, highest peak of DENV infection was observed in the month of October and September followed by November and least in the month of March, February, and May. These finding are similar to other studies [16], dengue infections were generally encountered in India during or after rains, as an outcome of rise in vector population. The febrile phase normally commenced during July or August and perpetuated till September or October. Highest number of epidemics occurred in the month of September and lowest between December and June. An exceptionally long-epidemic period was recorded in

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Calcutta where the haemorrhagic fever continued from July to March. Dengue haemorrhagic fever was induced by Group B Flavivirus DEN **2** in July which remained viable among the population specially children until October. Although other studies[17] also reported dengue epidemics during month of February, followed by April and March signify independent of rainfall and temperature, implying that factors for DENV transmission were spatially heterogeneous. Presence of DENV infection during dry month of March could be reflective of the year-round activity of the mosquito vector at domestic water supply, poor garbage control, plastic containers and automobile tires.

In the present study, 35% specimens were positive for NS1 antigen. Several studies have reported a lesser values of 16% (Shrivastava A et al, 2011) [18], 23.3% (Datta S et al, 2010)[19] , 30% (Kulkarni RD et al, 2011) [7] as well as higher value of even 60 % (SantoshTathe et al, 2013) [8] positivity exclusively for NS1 antigen for diagnosis of dengue. NS1 Ag assay is an effective tool for diagnosis of dengue infection, especially within the first four days of illness (Datta S et al, 2010) [19]. A recent meta-analysis for NS1-based test as a diagnostic utility for dengue infection supported the use of single NS1-based test with improved sensitivity of detection when combined with an IgM test (Zhang H et al, 2014)[20].

Dengue serotype diagnosed in present study was DENV 2(62.5%), DENV 1(12.5%) and mixed infection with DENV 2 and DENV 3(12.5%) & DENV 2+3+4(12.5%). The epidemiology of dengue fever in the Indian subcontinent has been very complex and has substantially changed over almost past six decades in terms of prevalent strains. The epidemiology of dengue virus and its prevalent serotypes has been ever changing. DENV-2 was the predominant serotype circulating in northern India, including Delhi, Lucknow and Gwalior [21,22,23] while DENV-1 was isolated during the 1997 epidemic at Delhi. The Gwalior DENV-2 viruses were classified into genotype-IV, and were most closely related to Delhi 1996 DENV-2. Co-circulation of several serotypes of dengue viruses has resulted in concurrent infection in some patients with multiple serotypes of DENV.

CONCLUSION

Dengue is the most important arboviral infection of public health significance. Dengue is reported at regular interval from different parts of India. In the present study investigation of patients suspected of having Dengue like illness was carried out during May 15 to April 16.

A total of 409 samples were screened and 100 samples are found positive for Dengue IgM antibody indicating a prevalence of 24.44% with maximum cases identified during month of Sept. and Oct. coinciding with postmonsoon period. 35 acute phase samples were found positive for Dengue NS1 antigen. The RT-PCR of these NS1 positive samples reviewed. 8 samples found to be positive for Dengue virus. Further serotyping by multiplex PCR indicating circulation of multiple serotypes of Dengue viruses in this region. DENV-2 found to be the predominant serotype followed by DENV-1 & 3. The study serves as the baseline data about circulation of Dengue viruses in Gwalior region. Regular continuous surveillance is warranted to monitor the circulation of Dengue virus in future.

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