Original Research Paper

Dengue biting again : A five year retrospective study in a tertiary care hospital

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Abstract:

Dengue fever is caused by an arbovirus worldwide, especially in the tropical and sub-tropical regions. Its clinical manifestations range from mild fever to a severe disease characterized by hemorrhage and shock. This is transmitted to humans by day-biting *Aedes aegypti* and *Aedes albopictus* mosquitoes.

Methods:

A total of 18637cases were detected in last five years (2014-2018). 5 ml blood sample was collected from the suspected cases of Dengue, which were tested by NS1 Antigen detection by NS1Ag ELISA and for IgM antibody by IgM MAC ELISA depending upon the duration of fever.

Results:

Out 0f 18637, 10447 cases were positive for Dengue infection. The infection was more in males as compare to females and was more common in the age group of 21-40 years (42.2%). The disease is more prevalent in urban areas (69%) as compare to rural areas(31%). It was revealed from the present study that the trend of dengue infection is keep on increasing with each successive year from 2014 to 2018.

Conclusion:

Dengue affects humans of all age-groups and causes high moratlity. Thus, a rapid and accurate dengue diagnosis is of paramount importance for effective control of dengue outbreaks.

Key Words:

Dengue Fever, Arbovirus, Hemorrhage, Shock, ELISA

Introduction

Dengue fever is the most important mosquito- borne arbo viral infection and is responsible for high mortality in humans. *It* is prevalent in most parts of the tropical and subtropical areas of Asia, Africa and Central and South America. The disease is transmitted to humans by day-biting *Aedes aegypti* and *Aedes albopictus* mosquitoes. Dengue virus occur as four antigenetically distinct serotypes infection with any of them leading from moderate febrile illness to severe form of disease i.e Dengue haemorrhagic fever -Dengue shock syndrome (DHF-DSS). [1,2]

Estimated annual incidence of disease caused by dengue virus (DENV) is 50–100 million cases of dengue fever and 2,50,000 cases of dengue haemorrhagic fever; mortality rate is 25,000 per year in tropical and subtropical countries. The first outbreak of dengue fever in India was in 1812. Several major outbreaks took place after this in years 1836, 1906, 1911, 1972, 2005, 2010, and 2015. [1]

There are about 390 million cases of dengue fever worldwide, and of the total number of cases, 96 million require hospitalization in 2016. Today, 40% of the world's population live in areas where there is a risk of dengue transmission.The World Health Organization (WHO) estimates that 50 to 100 million infections occur yearly, including 500,000 DHF cases and 22,000 deaths, mostly among children.[5]

Many outbreaks of dengue have been reported regularly from different parts of India since 1945. In recent years, the disease has been manifesting in severe form as DHF and with increasing frequency of outbreaks. In 1996 one of major outbreaks in North India occurred in Delhi and adjoining area, which was mainly due to Dengue -2 serotype. Where as in 2003 outbreak Dengue-3 serotype was found in North India. India also saw a doubling up of cases of dengue from 2014 to 2015 and the worst hit city was Delhi with over 1800 cases of the fever [5].

Morphology

The Dengue virus, virion comprises a spherical particle, 40–50 nm in diameter, with a lipopolysaccharide envelope. The RNA genome contains a single open reading frame and is approximately 10.7 kilobases in length. The genome encodes a precursor polypeptide in which post-translational cleavage by host cell and virus-encoded protease results in formation of three structural proteins—capsid (C), membrane (M), and envelop (E), and seven non-structural proteins- NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5. These structural and non-structural proteins play several functions in the life cycle and pathogenesis of DENV. E protein, is the principal surface protein of the virus particle (virion), and is involved in host cell attachment, especially to keratinocytes and is responsible for humoral immunity and mutation affects DENV virulence. M protein is important for virion formation and maturation, while the nucleocapsid is formed by C protein. Of the

nonstructural proteins, NS1 is a highly conserved glycoprotein that appears essential to pathogenesis. It is present in elevated concentrations in sera of dengue-infected patients during the early clinical phase of infection. However, detailed biological functions are yet to be ascribed. Other well characterised non-structural proteins are NS3 and NS5 which assist viral RNA synthesis and serve as an RNA-dependent RNA polymerase, respectively. There are five antigenically distinct serotypes of DENV, 1 - 5, the last one of which was discovered only very recently. Infection with one serotype will provide lifelong immunity to the exposed serotype but does not provide protection against others [3,4].

Life Cycle in Mosquito

The mosquito vectors, principally *Aedes aegypti*, become infected when they feed on humans during the usual five-day period of viraemia. The virus passes from the mosquito intestinal tract to the salivary glands after an extrinsic incubation period, a process that takes approximately 10 days and is most rapid at high ambient temperatures. Mosquito bites after the extrinsic incubation period result in infection, which might be promoted by mosquito salivary proteins [1].

Denv Pathogenicity

Replication of the dengue virus occurs within mononuclear cells including skin dendritic cells, tissue macrophages, peripheral blood monocytes, and hepatocytes. In the skin, dengue viruses infect immature dendritic cells through the non-specific receptor dendritic cell-specific ICAM3-grabbing non-integrin (DC-SIGN). Infected dendritic cells mature and migrate to local or regional lymph nodes where they present viral antigens to T cells, initiating the cellular and humoral immune responses. There is replication of DENVs in liver parenchymal cells and in macrophages in lymph nodes, liver and spleen, as well as in peripheral blood monocytes. Both *macrophages* and monocytes participate in antibody-dependent enhancement (ADE) [1]. The DEN viruses are closely related serologically but they are antigenically distinct. Primary or first infection in non immune persons usually causes DF. Subsequent dengue infection by a different serotype causes more severe illness, such as DHF/DSS. The key manifestations of DHF/DSS are sudden onset of shock, capillary leakage, and hemorrhagic diathesis/thrombocytopenia occurring at the time of defervescence of fever. [4,6,13].

Primary infection by one DEN virus serotype does not protect against infection from another DEN virus serotype. The antibodies raised against the primary infecting DEN virus serotype may cross-react with the subsequent infecting DEN virus serotype and through opsonization may function to enhance the ability of the second DEN virus serotype to infect the host, in a process called antibody-dependent enhancement of infection . The resultant higher viral antigen load leads to an exaggerated activation of cross-reactive dengue specific T cells. DHF and DSS are characterized by a diminished IgM antibody response that will promote infection of higher number of mononuclear cells, followed by the release of cytokines, vasoactive mediators that will increase the vascular permeability and haemorrhage leading to Disseminated Intravascular Coagulations (DIC) followed by vascular collapse, which may lead to death of the patient. This phenomenon is known as antibody-dependent enhancement. [4,6,13]

DENVs produce several syndromes that are conditioned by age and immunological status. During initial dengue infections, most children experience subclinical infection or mild undifferentiated febrile syndromes. During secondary dengue infections the pathophysiology of the disease changes dramatically, particularly sequential infections in which infection with DENV-1 is followed by infection with DENV-2 or DENV-3, or infection with DENV-3 is followed by infection with DENV-2 [2,4]. Such infections can result in an acute vascular permeability syndrome known as dengue shock syndrome (DSS). The severity of DSS is age-dependent, with vascular leakage being most severe in young children, a phenomenon that is thought to be related to the intrinsic integrity of the capillaries [5,6]. In adults, primary infections with each of the four DENV serotypes, particularly with DENV-1 and -3, often results in DF. Some outbreaks of primary DENV-2 infections have been predominantly subclinical. Nonetheless, dengue infections in adults are often accompanied by a tendency for bleeding that can lead to severe haemorrhages. [4]

Secondary dengue infections in adults can produce the classical DSS or severe disease complicated by haemorrhages. The severity of secondary dengue infections has been observed to increase from month-to-month during island outbreaks, the longer the interval between the first and second infection the more severe is the accompanying disease Tertiary dengue infections can cause severe disease, but only rarely. [7,8,9]

CLINICAL MANIFESTATIONS

Incubation period is 4 to 7 days (range 3–14 days).

Undifferentiated fever

This stage is seen mostly in the primary infection but may also occur following the initial secondary infection. Clinically, it is difficult to differentiate from numerous other viral diseases and often remains undiagnosed.

Dengue Fever

DF follows both primary and secondary infections, and is most frequently encountered in adults and older children. Onset of symptoms is characterized by a biphasic, high-grade fever lasting for 3 days to 1 week. It is associated with Severe headache (mainly retro bulbar), myalgia, joint pain, diarrhoea, vomiting and cutaneous rash. Dengue is also known as break bone fever because of the associated myalgia and pain in joints.

Dengue Hemorrhagic Fever

DHF is frequently seen during a secondary dengue infection. However, in infants it may also occur during a primary infection due to maternally attained dengue antibodies. The proposed diagnostic criteria for DHF includes:

Clinical parameters: Acute-onset febrile phase – high-grade fever lasting from 2 days to 1 week. Hemorrhagic episodes (at least one of the following forms): Petechiae, purpura, ecchymosis, epistaxis, gingival and mucosal bleeding, GIT or injection site, hematemesis and/or malena.

The haemorrhagic episodes in DHF are associated with multifactorial pathogenesis i.e platelet deficiency and dysfunction, defects in the blood coagulation pathways and Vasculopathy. Decreased production of platelets and increased destruction of platelets may result in thrombocytopenia in DHF. The impaired platelet function causes the blood vessels to become fragile and these results in haemorrhage.

The clinical course of DHF is characterized by three phases: High Grade Fever, plasma leakage, and convalescent phase. The initial febrile illness is marked by a morbilliform rash and haemorrhagic tendencies. The fever last for 2 -7days followed by either convalescent phase or to the plasma leakage phase. High plasma leakage lead to frank shock with bradycardia, cyanosis, hepatomegaly, pleural and pericardial effusions, and ascites. Pallor, severe ecchymosis and gastrointestinal bleeding followed by epistaxis may also be noted in a few cases.

Dengue Shock Syndrome

DSS is defined as DHF accompanied by a unstable pulse, narrow pulse pressure (<20 mmHg), restlessness, cold, clammy skin, and circumoral cyanosis. Progressively worsening shock, multi organ damage, and disseminated intravascular coagulation account for a high mortality rate associated with DSS. The shock persists for a short span of time and the patient promptly recovers with supportive therapy.

Objectives

To study the prevalence of Dengue fever, its diagnosis and its prevention.

Material And Methods

A retrospective study was performed at a tertiary care hospital of northern India for the period of 2014-2018. A total of 18637 serum samples from suspected dengue cases attending OPD or admitted in a tertiary care hospital of Punjab were tested for confirmation of Dengue. 5 ml blood sample was collected aseptically from patients who were clinically suspected of dengue fever and have any or all of- fever/headache/myalgia/retro orbital pain/rash/hemorrhagic manifestations in the acute phase of their illness and the sample was transported to Viral diagnostic laboratory, Department of Microbiology, GMC Patiala. If the Febrile patients has H/O fever less than 5 days, then the sample was tested for NS1Agby using NS1 Ag ELISA test kit (Panbio Diagnostics). If there H/O fever more than 5 days then the sample was tested for IgM antibody with IgM Mac Elisa test kit (National Institute of Virology, Pune,India).

ELISA tests were performed as per the manufacturer's instructions and results will be interpreted as positive, equivocal and negative.

Results

Out of 18637 serum sample of suspected cases which were collected during the period of 2014 to 2018. **10447** (56%) samples came out to positive for dengue viral infection either by NS1 antigen or for IgM antibody (Table-1). The dengue fever was more prevalent in 21-40 years (42.2%) of patients followed by 41-60 years (23.9%) and 0-20 years (23.3%) (Table -2).Maximum patients were from urban background (69%) as compare to rural background (31%) (Fig. 1). The trend of dengue infection keep on increasing with each successive year i.e 195 cases (2014), 4396 cases (2015), 4512 cases (2016), 5183 cases (2017) and 4351 cases (2018) (Table-1). The peak of dengue was seen in post monsoon i.e in September, October and November and they start declining in December (Fig-1). The clinical profile of dengue revealed that fever,

Table 1: Year wise distribution of positive cases

Year	Positive	Negative	Total tested
2014	33 (17%)	162 (83%)	195
2015	2661 (60.5%)	1735 (39.5%)	4396
2016	1806 (40%)	2706 (60%)	4512
2017	2832 (54.6%)	2351 (45.4%)	5183
2018	3115 (71.5%)	1236(28.5%)	4351
Total	**10447** (56%)	**8190** (44%)	**18637**

Table 2: Geographic distribution of confirmed positive dengue cases according to age and sex

S.No	Age group	Male			Female		
		Urban	Rural	Total	Urban	Rural	Total
1	0-20	1280 (74.5%)	436 (25.5%)	1716	484 (67%)	238 (33%)	722
2	21-40	1904 (60.3%)	1255 (39.7%)	3159	870 (69.3%)	385 (30.7%)	1255
3	41-60	1270 (70%)	553 (30%)	1823	488 (71.3%)	196 (28.7%)	684
4	61-80	406 (65%)	218 (35%)	624	216 (71.8%)	85 (28.2%)	301
5	>80	81 (91%)	8 (9%)	89	50 (67.6%)	24 (32.4%)	74
	Total	4941 (66.7%)	2470 (33.3%)	7411	2108 (69.4%)	928 (30.6%)	3036

Fig 1: Geographical distribution of suspected cases

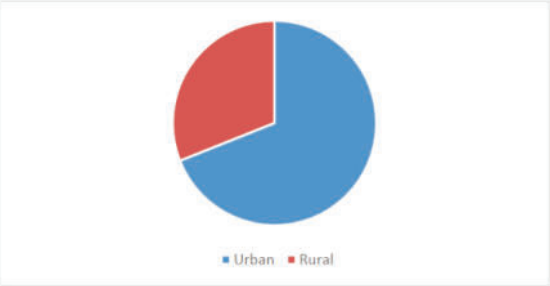
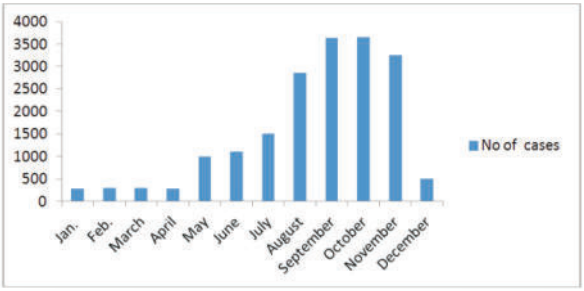


Fig -1 Seasonal Variation of Dengue cases from year 2014 to 2018



Discussion

Dengue is a major public health problem in India, involving almost all states since 2006, but in year 2013 extreme north was massively effected and in 2016 Punjab was among the worst hit states.

The present study is a retrospective study of the last five years from i.e 2014 - 2018. 18637 suspected cases of dengue fever were tested for dengue NS1 antigen or dengue IgM antibody ELISA test kits, out of which 10447 (56%) were came to be positive. The incidence of laboratory confirmed dengue was predominant in adults 21-40 years of age (42.2%) followed by 41-60 years (23.9%). Sex distribution shows a male preponderance(15.40%) in our study and this finding is in concordance with that of the earlier studies done by Tripathi et al, Neerja M, Baroor S and Darr L. [16,24,26,27]

Dengue has traditionally been held to be a disease of high population density tropical specially in urban areas.[17,18] But now a days increasing reports of dengue cases from rural areas were also reported from northern, southern and western India. [19,20,21,25,26,27, 28,29] The findings of the present study shows similar type of geographic picture with other parts of the country.

In the present study, seasonal prevalence of dengue viral infection was seen during the period from 2014- 2018. Dengue cases started in the beginning of July with the onset of pre-monsoon showers, cases were few until August and peak of cases were recorded during postmonsoon season i.e in September, October, November and which started declining in December. The trend of distribution of cases in the present study, during the period of study (2014-2018) was similar to other states in India as per the NVBDCP data, various studies conducted in India and in the neighbouring country Pakistan. It was also observed that along with increase rate of dengue transmission in the postmonsoon season there is higher rate of dengue positivity.[13,15,16, 25,26,27,28,29]

Headache and Myalgia were the most common presenting symptom, **10447** (100%) and rash with thrombocytopenia in 1112 (10.6%) cases.

Classical DF is characterized by sudden onset of high grade fever, accompanied by headache, retro-orbital pain, myalgia, and thrombocytopenia. In our study, the clinical profile of dengue revealed that fever, Headache and Myalgia were the most common presenting symptom, **10447** (100%) and rash with thrombocytopenia were seen in 1112 (10.6%) cases. Thus, most of dengue cases were clinically indistinguishable from other febrile illnesses, and could be missed lacking the clinical suspicion and timely diagnosis. The same was reported by the other authors in Northern part of India [9,10, 25,26,27,28,29]

Conclusion

DF is a common acute febrile illness which comes as an epidemic in various parts of the country including Punjab. Over the recent years, it has emerged as one of the dreaded fevers in the region. From this study, it is concluded that, dengue is prevalent during the postmonson season all over the country. Early dengue surveillance and control at source should be enhanced before the monsoon. There should be awareness and active participation of local population is required to control the disease. Early detection by using IgM Mac Elisa and NS1Ag ELISA in a case of fever of unknown origin, can decrease the mortality rate, especially during the local dengue transmission season.

Conflicts of Interest : None

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