“ROLE OF NS1 ANTIGEN DETECTION IN EARLY DIAGNOSIS OF DENGUE VIRUS INFECTION”

REG NO. BG0111004

Dissertation

Submitted to the
KLE University, Belgaum, Karnataka

In Partial Fulfillment
of the requirements for the degree of

M. D.
in
GENERAL MEDICINE

DEPARTMENT OF MEDICINE,
JAWAHARLAL NEHRU MEDICAL COLLEGE,
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ENDORSEMENT

This is to certify that the dissertation entitled **"ROLE OF NS1 ANTIGEN DETECTION IN EARLY DIAGNOSIS OF DENGUE VIRUS INFECTION"** is a bonafide research work done by **THE CANDIDATE REG NO. BG0111004**.

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LIST OF ABBREVIATIONS USED

Ab	-Antibody
AD	-Anno Domini
Ag	-Antigen
ALT	-Alanine aminotransferase
ARDS	-Acute Respiratory Distress Syndrome
AST	-Aspartate aminotransaminase
BUN	-Blood Urea Nitrogen
C3a	-Complement 3a
C5a	-Complement 5a
CD4	-Cluster of Differentiation
cmm	-cubic millimeter
DALYs	-Disability Adjusted Life Years
DF	-Dengue fever
DHF	-Dengue Hemmorhagic Fever
DIC	-Disseminated Intravascular Coagulation
dl	-deciliter
DSS	-Dengue Shock Syndrome
DV/DENV	-Dengue vírus
E	-Envelope
ECG	-Electrocardiography
ELISA	-Enzyme Linked Immuno Sorbent Assay
FDP	-Fibrin Degradation Product
GGT	- gamma-glutamyl transpeptidase
HAI	- Haemagglutination inhibition

Hct	-Haematocrit
ICT	- Immunochromatographic test
IgA	-Immunoglobulin A
IgE	-Immunoglobulin E
IgG	-Immunoglobulin g
IgM	-Immunoglobulin M
IL	-Interleukin
IU	-International unit
Kg	-kilogram
L	-liter
mAbs	-Monoclonal antibodies
MAC-ELISA	-Immunoglobulin M Capture ELISA
meq	-miliequivalence
mg	-miligram
ml	-Milliliter
Na+	- Sodium
NAAT	- Nucleic acid amplification test
NPV	-Negative Predictive Value
NS	-Non structural
NS	-Normal Saline
ORF	-Open Reading Frame
p value	- Probability value
PCR	-Polymerase Chain Reaction
PPV	-Positive Predictive Value
PRNT	- Plaque reduction neutralization test

PT	-Prothrombin time
PTT	-Partial Thromboplastin Time
RDT	-Rapid Detection Test
RL	-Ringers Lactate
RNA	-Ribonucleic acid
RT-PCR	-Reverse Transcriptase – Polymerase Chain Reaction
SD	- Standard deviation
SGOT	- Serum glutamic oxaloacetic transaminase
SGPT	- Serum glutamic pyruvic transaminase
Sr	-Serum
USG	-Ultrasonography
USG	-Ultrasonography
WBC	-White Blood Count
WHO	-World Health Organization

ABSTRACT

Background

Dengue fever is one of the most common tropical diseases worldwide. Early diagnosis of dengue helps in patient triage and timely management of dengue virus infection.

Objectives

This study was undertaken to study the early clinical features supported by detection of NS1 antigen for diagnosing dengue virus infection.

Methodology

The present one-year cross sectional study was conducted in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum. A total of 100 adult patients presenting with clinical features of dengue infection from January 2012 to December 2012 were studied. The diagnosis was confirmed with NS1 RDT whose efficacy was later tested with IgM ELISA.

Results

In this study young males were predominantly affected. NS1 positivity was 68%. The sensitivity of NS1 in predicting dengue infection compared to IgM was 92.86% and specificity was 90% with strength of agreement considered to be 'very good' based on Kappa statistics. Clinical features like retro orbital pain, myalgia, arthralgia, rashes and bleeding manifestations were significantly associated with NS1 positivity. Similarly icterus, edema, hypotension and altered

sensorium were significantly more in NS1 positive patients. SGOT more than SGPT, thrombocytopenia and raised creatinine were significantly more in NS1 positive patients.

Conclusion

Thus we conclude that dengue infection which possesses serious public health problem, can be diagnosed early with the help of clinical features supported by NS1 antigen rapid detection test can help in aiding early diagnosis.

Keywords

Clinical features, Dengue infection, NS1 RDT.

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Chapter 1

Introduction

INTRODUCTION

Arboviruses represent a serious public health problem. They are frequently associated with epidemics that have great economic and social impact in tropical and subtropical regions of the world.[1]

Dengue virus (DENV) causes a highly infectious illness and is transmitted to humans by mosquitos of the *Aedes* family causing high rates of morbidity and mortality.[1]

Dengue fever is the most important arthropod-borne viral infection of humans. Worldwide, an estimated 2.5 billion people are at risk of infection, approximately 975 million of whom live in urban areas of tropical and sub-tropical countries like Southeast Asia, the Pacific and the Americas.[2] The rural areas are also being increasingly affected in regions of Africa and the eastern Mediterranean. It is estimated that more than 50 million infections occur each year, of which 500,000 hospitalizations are of dengue haemorrhagic fever, mainly among children, with the case fatality rate exceeding 5% in some areas.[2-5]

Dengue fever (DF) has become a prominent infectious disease with outbreaks in many parts of the world. DF epidemics have reached almost 120 countries and in many of these countries it has a high incidence.[6]

According to historical accounts dengue fever emerged from Africa almost 500 to 600 years ago, and the first outbreaks reached different parts of world such as Asia and South America concurrently in the 1780s.[7]

During recent decades DF has become the second most prevalent mosquito-borne infection after malaria. Cases of DF have reached 50 million, while cases of Dengue hemorrhagic fever (DHF) are touching a staggering several hundred thousand per year. The most endemic regions include Latin America, Asia, and the Caribbean. Many dengue infections in travellers remain asymptomatic; a more serious issue is the travel-related spread of a more virulent dengue virus strain in an area with a less virulent strain, and also the spread of the virus in non-endemic regions with a high vector *(Aedes aegypti* and *Ae. albopictus)* population.[8-10]

The first outbreak of dengue fever in India was recorded in 1812.[11] In spite of preventive measures taken by the respective governments since then, recurrent outbreaks have occurred, and over the last 10 to 15 years DF has been the major cause of hospitalization and mortality after acute respiratory and diarrhoeal infections among children.[12] New Delhi, the capital of India located in the northern region of the country, experienced seven major outbreaks between 1967 and 2003.[13,14] Then in 2006 another major outbreak occurred with more than 11,000 reported cases and 165 reported fatal cases.

Dengue virus (DENV) belongs to the genus *Flavivirus* and the *Flaviviridae* family. It is an enveloped virus and contains a single stranded positive sense RNA of about 11kb and is classified in four serotypes designated as DENV-1, DENV-2, DENV-3 and DENV-4, based on its antigenic characteristics. The genome of DENV contains 10 genes in an open reading frame (ORF), the translation of the ORF results in a polyprotein that is processed by a signal-peptidase of the host cell into 3 structural proteins (C, E and pr M)

and 7 nonstructural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5). Infection by any of the above serotypes causes clinical manifestations that vary and are both nonspecific and benign, until more serious stages which sometimes have fatal consequences in the form of dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS).[15]

During the early febrile stage (the symptoms of which include fever, malaise, headache, body pains and rash), clinicians cannot predict which patients will progress to severe disease. Later, during defervescence, symptoms such as bleeding, thrombocytopenia of <100,000 platelets mm^{-3}, ascites, pleural effusion, increase in haematocrit of >20% and clinical warning signs, such as severe and continuous abdominal pain, restlessness and/or somnolence, persistent vomiting and a sudden reduction in temperature (from fever to subnormal temperature) associated with profuse perspiration, adynamia (loss of strength or vigor) and sometimes fainting, can be indicative of plasma extravasation and the imminence of shock. Other complications include massive haemorrhage, disseminated intravascular coagulation, multiple organ failure, and respiratory failure due to non-cardiogenic pulmonary oedema.[16-19]

More recently a cross-sectional study from Lucknow[20] during the monsoon and post-monsoon seasons in the year 2010 on 356 patients with suspected dengue fever found 138 (39%) had serologically confirmed dengue infection. Ninety-six (70%) patients had classical dengue fever while 42 (30%) had dengue hemorrhagic fever. The most common symptoms were headache (105, 76%), abdominal pain (87, 63%), vomiting (80, 58%), rash (36, 26%) and cutaneous hypersensitivity (22, 16%). Hemorrhagic manifestations were present

in 55 (40%) patients. Notably, 14% of patients had neurological involvement and 4% had acute hepatic failure.

Dengue fever has wide spectrum of clinical manifestations. Hence early laboratory confirmation of dengue infection is crucial. Among the methods available for dengue diagnosis, virus isolation provides the most specific test result. However, facilities that can support viral culture are not always available.

Detection of IgM or IgG antibodies is the standard for serologically confirming a dengue infection. The presence of IgM or high levels of IgG in acute serum collected from a suspected dengue case suggests a probable dengue infection.[16,17] The confirmation of the dengue infection is done by virus isolation, genome detection, antigen detection and IgM or IgG seroconversion. However, the probable dengue infection is done by IgM positive and elevated IgG titre (that is, 1,280 or greater by haemagglutination inhibition test).

In view of the high mortality rate and to reduce the disease burden, it is imperative to have a rapid and sensitive laboratory assay for early detection of the disease. The major diagnostic methods currently available are viral culture, viral RNA detection by reverse transcriptase PCR (RT-PCR) and serological tests such as an immunoglobulin M (IgM) capture enzyme-linked immunosorbent assay (MAC-ELISA).

However, early dengue diagnosis still remains a problem, as all these assays have their own pitfalls. The first two assays have restricted scope as a routine diagnostic procedure. Viral isolation by cell culture and subsequent detection by immunofluorescence, though the gold standard, cannot be used as a

routine diagnostic procedure due to its low sensitivity, laborious procedure and time consumption. The requirement of a highly trained staff, the need of a sophisticated equipment as well as the cost factor associated with molecular methods has limited its application as a routine diagnostic assay. The MAC-ELISA, which is a commonly used assay, has a low sensitivity in the first four days of illness. The requirement of paired sera at acute and convalescent phase, which improves the accuracy of the diagnosis, further delays it.[21]

Recently, an up-to-date test for early diagnosis of dengue infection is dengue NS1 antigen detection. NS1 is a glycoprotein produced by all flaviviruses and is essential for viral replication and viability. Because this protein is secreted into the bloodstream, many tests have been developed to diagnose DENV infections using NS1. These tests include antigen-capture ELISA, lateral flow antigen detection and measurement of NS1-specific IgM and IgG responses. NS1 antigen detection kits are now commercially available.[22]

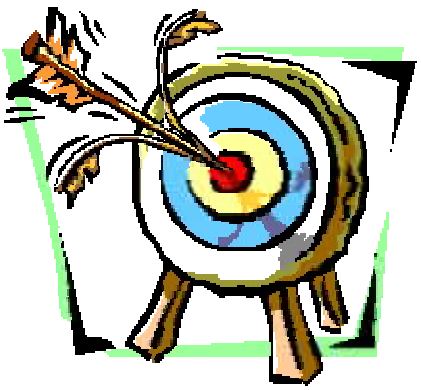
NS1 (non-structural protein 1) is a highly conserved glycoprotein that is essential for the viability of dengue virus (DENV) and is produced both in membrane-associated and secretory forms by the virus. NS1 antigen (NS1 Ag) have demonstrated its presence at high concentrations in the sera of DV infected patients during the early clinical phase of the disease. The detection of secretory NS1 protein represents a new approach to the diagnosis of acute DV infection.[21]

Evaluation of the NS1 assay indicated moderately high sensitivity and very high specificity to dengue infection. Also NS1 test is cost effective and provides results earlier. However, NS1 being the newer test very few studies have

reported the role of NS1 antigen detection in the early diagnosis of dengue virus infection and so far no such study has been done in North Karnataka. Hence the present study was undertaken to find out the role of NS1 antigen for early detection of dengue virus infection versus ELISA and to study the clinical features in early stage of dengue infection.

Chapter 2

Objectives



OBJECTIVES

The objectives of the present study were;

1. To find out the role of NS1 antigen for early detection of dengue virus infection versus IgM ELISA.

2. To study the clinical features in early stage of dengue infection.

Chapter 3

Review of Literature

REVIEW OF LITERATURE

Historical Aspects

The term "Dengue" was coined in the English medical literature from the West Indies during the 1827–1828 Caribbean epidemic that presented as exanthema with arthralgia. Dengue is a Spanish synonym for the Swahili "Ki denga Pepo" (a sudden cramp like seizure caused by an evil spirit). The term "Break bone fever" for the modern dengue was proposed in Philadelphia in 1780.[23] *Aedes aegypti* mosquito as a vector of dengue virus was first discovered by Bancroft in 1906.[24]

Isolation and detection of dengue virus dates back to World War II and further characterization led to postulation of dengue virus as an agent involved in various past outbreaks which were exhibiting dengue-like symptoms. Dengue-like disease was mentioned in ancient Chinese manuscripts dates back to 992 A.D. and to the 1600s A.D. in the West Indies.[24]

The first historical account of DSS was reported by Benjamin Rush during an outbreak (1780) in Philadelphia among people living at Delaware River.[25] North America had similar outbreaks in the 18th and 19th centuries along the Atlantic coast, on the Caribbean Islands, and also in the Mississippi basin.

Shock and death cases were documented in a dengue epidemic in Queens land, Australia in 1897, while nearly 1250 persons died during the explosive Greek dengue epidemic of 1928. Another epidemic was related to substandard

living conditions among refugees who moved from Turkey following the Greco-Turkish war of 1922. Dengue viruses for the first time were adapted to laboratory animals in the 1940's (Dengue type 1 and 2) and 1950's (Dengue type 3 and 4).[23]

However, it was only in 1943-44 that the modern chapter of dengue research started. This was when for the first time dengue virus was cultured and later isolated from suckling mice brain.[24]

Dengue fever was first observed in Africa, but later with the increase in trade DF reached all parts of the world including Asia, South America and the Indian subcontinent that includes India, Pakistan, Bangladesh and Sri Lanka. These countries experience epidemics every year with cases reaching several thousands in numbers.[24]

Hammon et al in 1956 mentioned that DHF/DSS were associated with dengue virus.[23]

The first case of dengue hemorrhagic fever in Southeast Asia was noted in Manila in 1953 to 1954 and outbreaks have since then been reported throughout the Indo-China Peninsula and the Indian sub-continent.[26]

Dengue virus belongs to the Arbovirus group of viruses that are transmitted through insect vectors most commomly Aedes Aegypti mosquito. Virions are 40-50 nm in diameter and spherical in shape with 11kb single-stranded RNA containing a single open reading frame. Dengue virus consists of ten proteins, three of which are structural and seven non structural, and it has four serotypes, namely DENV1, DENV2, DENV3 and DENV4.[24]

The Indian subcontinent is mainly affected by DENV2 and DENV3 serotypes. DENV1 and DENV4 were identified by studying neutralizing antibodies in the blood of volunteers in 1973 while DENV1 and DENV2 were isolated as a consequence of the failure of viral strains to cross-protect human volunteers.[27]

All four virus serotypes cause similar illness, but severe and fatal hemorrhagic disease is more often associated with DENV2 and DENV3 infections.[24] DENV2 (genotype IV) and DENV3 (genotype III) are the most commonly isolated genotypes.[24]

Epidemic and pandemic of dengue virus infections[28]

Benjamin Rush identified the tip of the 18th century pandemic from the classic description of dengue fever in Philadelphia in 1780. The causal virus and mosquito were introduced into Philadelphia by ship, an unwelcome consequence of the sugar, rum and slave trade between African, colonial American and Caribbean ports. This first pandemic produced reports of sporadic dengue outbreaks in the United States of America, Caribbean and South American coastal cities during the 19th century and first three decades of 20th century. Second pandemic occurred in semitropical Northern Queensland.

The 20th century pandemic increased after World War II in which soldiers from South East Asia to Japan and Pacific Islands carried dengue strains. Destruction of city water supplies, temporary housing for refugees, the explosive post – war growth of populations through high fertility, rural to urban migration and the steady decline of urban environments, have led to steady growth in

density and the area occupied by Aedes aegypti. Together these factors have resulted in the endemic transmission of all four dengue serotypes in most of the Asian tropics.

Mean while, the remarkable gains achieved towards the eradication of Aedes aegypti in the American tropics have been eroded and reversed. This was followed by the introduction and spread of dengue viruses beginning in the 1960s. Dengue viruses have invaded Cuba, Caribbean Islands, Mexico, the United States, Central America, Colombia, Ecuador, Peru, Paraguay, Bolivia, Argentina and Brazil. By the 1990s dengue had spread to China, Taiwan, south to Australia and eastward to nearly all of the Pacific Islands. In Africa and the Middle East, areas of epidemic activity include outbreaks in Kenya. Mozambique, Somalia and Yemen. Major recent outbreaks occurred in Cuba (1981), Southern China, Sri Lanka, India, Maldives, Tahiti and Venezuela in mid to late 1980s.

Epidemics of dengue fever in India[29]

Dengue fever is endemic in many parts of India barring the Himalayan and other mountainous regions where conditions are not conductive to the propagation of its vector. Outbreaks of dengue fever occur mostly in India, during or after the rainy season, but outbreaks during summer season have also been reported due to storage of water for domestic purposes causing a rise in vector population.

Some of the epidemics of DHF/DSS which occurred in India are as follows:[30]

Year	Region	Type of dengue virus
1964	Vellore, Tamil Nadu	DV-2
1966	Vellore, Tamil Nadu	DV-3
1968	Vellore, Tamil Nadu	DV- 1,2,3 & 4
1968	Kanpur, Uttar Pradesh	DV-4
1969	Kanpur, Uttar Pradesh	DV-4 and DV-2
1970	Hardoi, Uttar Pradesh	DV-2
NA	NA	DV- 1,2,3 & 4
1983	Kolkata, West Bengal	DV-3
1985	Jalore town, South-West Rajasthan	DV-3
1988	Delhi	DV-2
1990	Calcutta, West Bengal	DV-3
1988	Rural and urban areas of Gujarat	DV-2
1993	Mangalore, Karnataka	DV-2
1996	Ludhiana, Punjab	DV- 1,2,3 & 4
1996	Lucknow	DV-2
1996	Delhi	DV-2
1996	Delhi	DV-2
1997	Delhi	DV-1
1996	Delhi	DV-2 (Genotype IV)
1997	Delhi	DV-1
1996	Rural areas of Haryana	DV-2
2001	Dharmapuri district, Tamil Nadu	DV-2
NA	Andaman and Nicobar Islands	DV-2
2001	Gwalior, Madhya Pradesh	DV-2
2001	Chennai, Tamil Nadu	DV-3
2003	Northern India (Delhi & Gwalior)	DV-3
2005	Kolkata, West Bengal	DV-3
2003	Kanyakumari district, Tamil Nadu	DV-3
2003-04	Delhi	DV-3 (subtype III)
2003-05	Delhi	DV-1,2,3 & 4
2006	Delhi	DV-3
2006	Delhi	DV-1 & 3
2001-07	North India (Delhi and Gwalior region)	DV-1 (Genotype III)
2006	Delhi	DV-1,3 & 4
2008	Delhi region	DV-1,2 & 3
1956-2005	Entire country	DV-2
2002-06	Delhi	DV-1, 2, 3 & 4
2003	Delhi	DV-3 (Genotype III)
2008	Ernakulam, Kerala	DV-2 & 3
2007	Rural areas of Madurai, Tamil Nadu	DV-3 (Genotype III)
2007	Andhra Pradesh	DV-1 & 4 (Genotype I)
2003-08	Different parts of the country	DV-3 (Genotype III)
2007-09	Delhi	DV 1, 2, 3 & 4
2009-10	Pune, Maharashtra	DV-4 (Genotype I)

Epidemiology

Worldwide

Dengue is the most important arthropod-borne viral infection of humans. Worldwide, an estimated 2.5 billion people are at risk of infection, approximately 975 million of whom live in urban areas in tropical and sub-tropical countries in Southeast Asia, the Pacific, the Americas[1], Africa, the Eastern Mediterranean, and rural communities are increasingly being affected. It is estimated that more than 50 million infections occur each year, including 500,000 hospitalizations for dengue hemorrhagic fever, mainly among children, with the case fatality rate exceeding 5% in some areas.[22]

The annual average number of dengue fever/dengue hemorrhagic fever (DF/DHF) cases reported to the World Health Organization (WHO) has increased significantly in last few years. For the period 2000–2004, the annual average was 925,896 cases; almost double the figure of 479,848 cases that was reported for the period 1990–1999. In 2001, record 69 countries reported dengue activity to WHO and in 2002, the Region of the Americas alone reported more than one million cases. Although there is poor surveillance and no official reporting of dengue to WHO from countries in the African and Eastern Mediterranean regions, in 2005–2006 outbreaks of suspected dengue were recorded in Pakistan, Saudi Arabia, Yemen, Sudan and Madagascar, [1-4] and a large outbreak of dengue involving >17,000 cases was documented in the Cape Verde islands in 2009.[31]

Travellers from endemic areas might serve as vehicles for further spread. Dengue epidemics can have a significant economic and health toll. In endemic

countries in Asia and the Americas, the burden of dengue is approximately 1,300 disability-adjusted life years (DALYs) per million population, which is similar to the disease burden of other childhood and tropical diseases, including tuberculosis, in these regions.[22]

The geographical areas in which dengue transmission occurs have expanded in recent years, and all four dengue virus serotypes (DENV-1–4) are now circulating in Asia, Africa and the Americas, a dramatically different scenario from that which prevailed 20 or 30 years ago.[32]

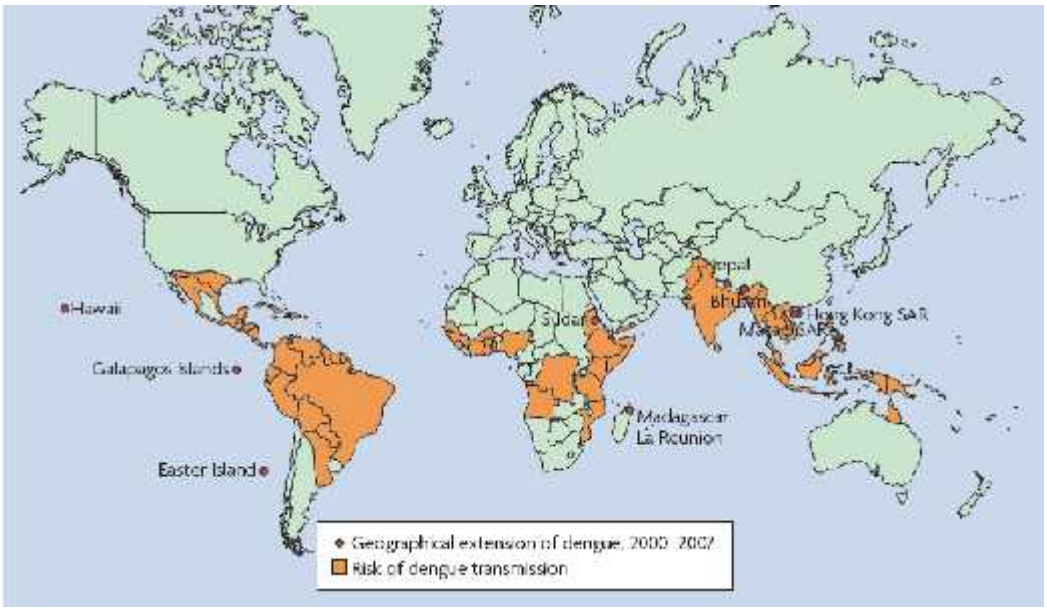


Figure 1. Countries and areas at risk of dengue transmission, 2007[22]

Indian scenario

The first outbreak of dengue fever in India was recorded in 1812.[11] In spite of preventive measures taken by the respective governments since then, recurrent outbreaks have occurred, and over the last 10 to 15 years DF has been

the major cause of hospitalization and mortality after acute respiratory and diarrheal infections among children.[12]

New Delhi, the capital of India located in the northern region of the country, experienced seven major outbreaks between 1967 and 2003.[13,14]

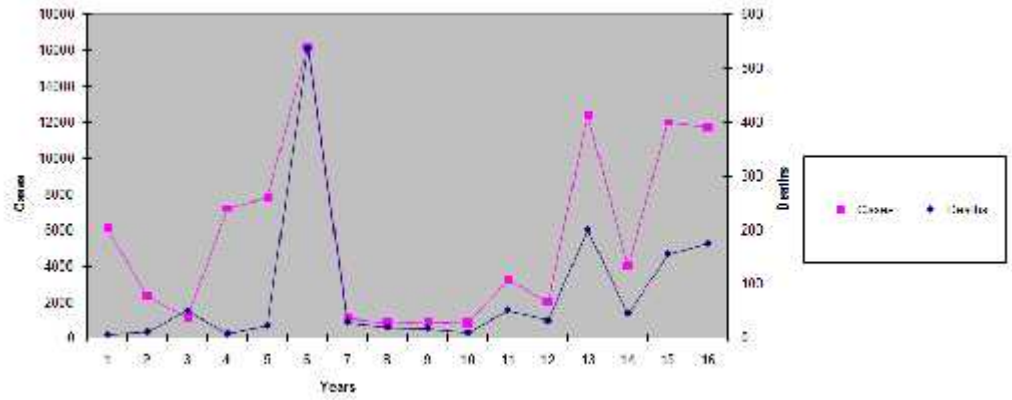


Figure 2. DF reported cases and deaths in India from 1991 till 2008[24]

Then in 2006 another major outbreak occurred with more than 11,000 reported cases and 165 reported fatal cases. Figure 2 shows data obtained from the World Health Organization (WHO) exhibiting the number of DF cases reported in India from 1991 to 2008 as well as the annual reported fatality rate during this period.[33]

Samples isolated from Gujarat state showed that the epidemics of 1988-89 were dominated by DENV2. As time passed, dengue virus outbreaks reached different states of India. In 1992 Jammu also saw an outbreak of DENV2 followed by an outbreak in Haryana. DENV2 outbreaks were seen in Northern India in Delhi, Lucknow and Gwalior. However, DENV1 was the predominant serotype in the outbreak of 1997 in New Delhi. The Gwalior outbreaks of 2003-

04 were dominated by DENV3, and DENV3 was also prominent in 2004-05 at Delhi. This co-circulation of serotypes in the same area might be the reason behind the large number of DHF cases reported that year.[30]

Rapid growth of the population and sudden climatic changes contributed to the increase in cases of DF/DHF in India. During 1997 until 2004, DENV1 was seen as the causative agent of most DF/DHF cases but later in 2005, DENV3 became the leading source of dengue outbreaks. According to the WHO in 2006, the total number of reported cases reached 12,317, while in 2007 fewer cases occurred 5,534 owing greatly to preventive measures taken by both the public and private sectors. In 2009, however, DF cases again reached 11,476 by November. Initial cases were reported in July 2009 with the greatest number of cases seen in October. These trends demonstrate that DENV has penetrated deep into India, with DENV2 and DENV3 predominating among different DENV serotypes.[24]

Etiology

Arboviruses (Arthropod borne viruses) are viruses of vertebrates, biologically transmitted by hematophagous insect vector. They multiply in blood sucking insects and are transmitted by bite to vertebrate hosts. Taxonomically, Arboviruses belong to families as diverse as Togaviridae, Bunyaviridae, Reoviridae, Arenaviridae and Rhabdoviridae.

Togaviruses are spherical viruses, 40-70 nm in diameter with lipoprotein envelope and single stranded RNA genome. The Togavirus family contains three genera of medically important viruses.[34]

Alphavirus : Arbovirus Group A

Flavivirus : Arbovirus Group B. The name being derived from flavi meaning yellow

Rubivirus : Rubella virus. It is antigenically and epidemiologically unrelated to arboviruses

Dengue virus, a species of flavivirus genus belonging to Togaviridae family, is a single stranded RNA virus. There are four serotypes of dengue virus, which are 1, 2, 3 and 4, and all serotypes can cause DF and DHF. All four types of dengue viruses have been isolated in this country and occasionally more than one type of dengue virus have been isolated from the same patient. The virus can survive at 4°C for several weeks and at 70°C for years.

Transmission and vector

The mosquitoes of "Stegomyia family" transmit dengue viruses. Aedes aegypti is the principal vector. Other vectors, which are also responsible for outbreaks of dengue infections are Aedes albopictus, Aedes polynesiensis and Aedes scutellaris complex.[35]

These are domestic mosquitoes and are most abundant during the rainy season. Females are fearless biters and bite during daytime. Two peak biting activity periods being two to three hours after dawn and in the afternoon few hours before dark. They do not fly over long distances and epidemic transmission of dengue requires a favorable temperature (>20°C).

The reservoir of infection is both man and mosquito. The transmission cycle is “man-mosquito-man”, although in jungle setting, probably the monkeys are also responsible for maintaining this infection cycle. The Aedes mosquito becomes infective by feeding on a patient during viremia i.e. from a day before onset to the fifth day of illness. The virus multiplies in its salivary glands. After an incubation period of 8 to 10 days the mosquito becomes infective and is able to transmit the disease. Once the mosquito become infective, it remains so for life.

Pathology and Pathogenesis

Certain terms, which are come across during the pathogenesis of dengue fever are;

Homotypic infection

Refers to the infection caused by dengue virus strains of a single serotype.

Heterotypic infection

Refers to the infection caused by different virus serotype.

Primary infection

Is infection caused by any serotype in non-immune individual.

Secondary infection

Is heterotypic infection in a monotype immune individual.

Tertiary infection

Is heterotypic infection in a multitypic immune individual (two infection).

The most significant pathophysiologic changes among DV infections are seen in DHF/DSS, due to plasma leakage from intravascular to extravascular compartments. The leakage of plasma leads to hemoconcentration, hypotension, hypoproteinemia and collection of fluid in serious cavities. The plasma leakage occurs as a result of acute increase in vascular permeability, which is attributed to transient functional disturbance due to action of short acting chemical mediators as no significant inflammatory or destructive vascular lesions are seen on histological examination.

Most accepted hypothesis explaining the pathogenesis of DHF/DSS is immune enhancement hypothesis. According to this hypothesis presence of non-neutralizing heterologus antibody is necessary for occurrence of serious manifestations due to vessel wall dysfunction. This heterologous antibody acquired either transplacentally from mothers or as a result of first infection binds to DV and facilitate the entry of virus into the cells of monocyte macrophage lineage. Within these cells, rapid viral replication occurs through a process called antibody dependent enhancement. These cells produce various vasoactive mediators e.g. tumour necrosis factor, interleukins (IL-1, IL-2, IL-6 etc.), platelet activating factor, complement activation products (C3a, C5a) and histamine. Simultaneously CD4 + T-Lymphocytes are also induced to produce gamma interferon, lymphotoxins and various interleukins. These cytokines have a

complex interplay and act synergistically on vessel wall to produce increased vascular permeability.[36]

Though immunopathogenesis is important in the severity of DHF/DSS, certain viral factors may also be important determinant of severity, genetic changes might be occurring in the virus leading to variation in virulence and epidemic potential. Certain host factors like age, state of nutrition, sequence of infection for example serotype 1 followed by serotype 2 is more dangerous than serotype 4 followed by serotype 2 are also important in determining the severity of disease.[37]

There are four serologically related dengue viruses that parenterally enter human hosts. After a short period of cross protection, individuals infected with one serotype are fully susceptible to infection with other types; in contrast there is life long immunity to reinfection by the homologus serotype. Primary and heterologous infections can be distinguished by their characteristic serological responses. In primary dengue infections antibody responses are largely of IgM class and predominantly directed against type specific determinant. In secondary infections antibodies are largely of IgG class and directed against the antigens of flavivirus group on the dengue virus complex or sub complex.[38] Three major hemostatic factors appear to be involved in the bleeding diathesis in DHF/DSS, which are; [39]

Vascular injury

Vasculopathy is manifested by petechiae, positive tourniquet test and leakage of fluid and protein into extravascular spaces. This cause an acute

increase in vascular permeability leading to loss of plasma from the vascular compartment, clinically producing pleural effusion, ascites, hemoconcentration, hypoproteinemia and shock. It is said that chemical mediators, histamine and not endothelial infection generate vascular permeability.

Coagulopathy

Weiss and Halstead et al observed a moderate prolongation of the prothrombin time due to decrease in factors II, V, VII and X.

In WHO collaborative study, platelet counts and average minimum fibrinogen level fell in correlation with severity of illness, while fibrin degradation products (FDP) rose correspondingly.

Suratte et al, Bokish et al and Srichaikul et al confirmed the mild increase in FDP but since euglobulin clot lysis times were normal, the authors concluded that there is evidence of mild to moderate consumptive coagulopahty, but no DIC, also it contributes neither to shock nor to bleeding nor was therapy with heparin justified.

Thrombocytopenia

The cause of thrombocytopenia is controversial, but the possibilities include impaired megakaryocyte production earlier in the disease, platelet injury by virus itself, platelet specific antibodies, immune complexes or DIC.

Hematological abnormalities[39]

In DF, leukopenia begins on day two of infection, reaching low point on fourth to sixth day along with early absolute neutropenia and lymphopenia, gradually returning to normal by ninth to tenth day with lymphocyte count returning to normal before neutrophils. Contrast to it in DHF/DSS cases where early absolute leukopenia was observed, in a few cases moderate leukocytosis between days four to nine along with early relative lymphocytosis is observed. In both syndromes there occurred marked degeneration of mature neutrophils and "shift to left" during febrile phases of illness. Atypical or transformed lymphocytes were seen on day fifth of illness, which have large nuclei with fine, homogenous nuclear chromatin and azurophilic cytoplasm. A bone marrow biopsies on fourth day of fever in dengue fever found that the bone marrow was hypocellular with diminished megakaryocytes, diminished erythropoiesis and totally absent granulocytopoiesis, on day seven and ten the bone marrow cellularity returns to normal. In DHF/DSS, early in febrile course, the bone marrow is hypocellular with maturation arrest of all elements. At the time of shock or defervescence marrow are usually normocellular or hyper-cellular with an unusual incidence of phagocytic reticulum cells.

Clinical features

The dengue virus infection may be manifested as asymptomatic to symptomatic disease as classical dengue fever, dengue hemorrhagic fever/dengue shock syndrome.

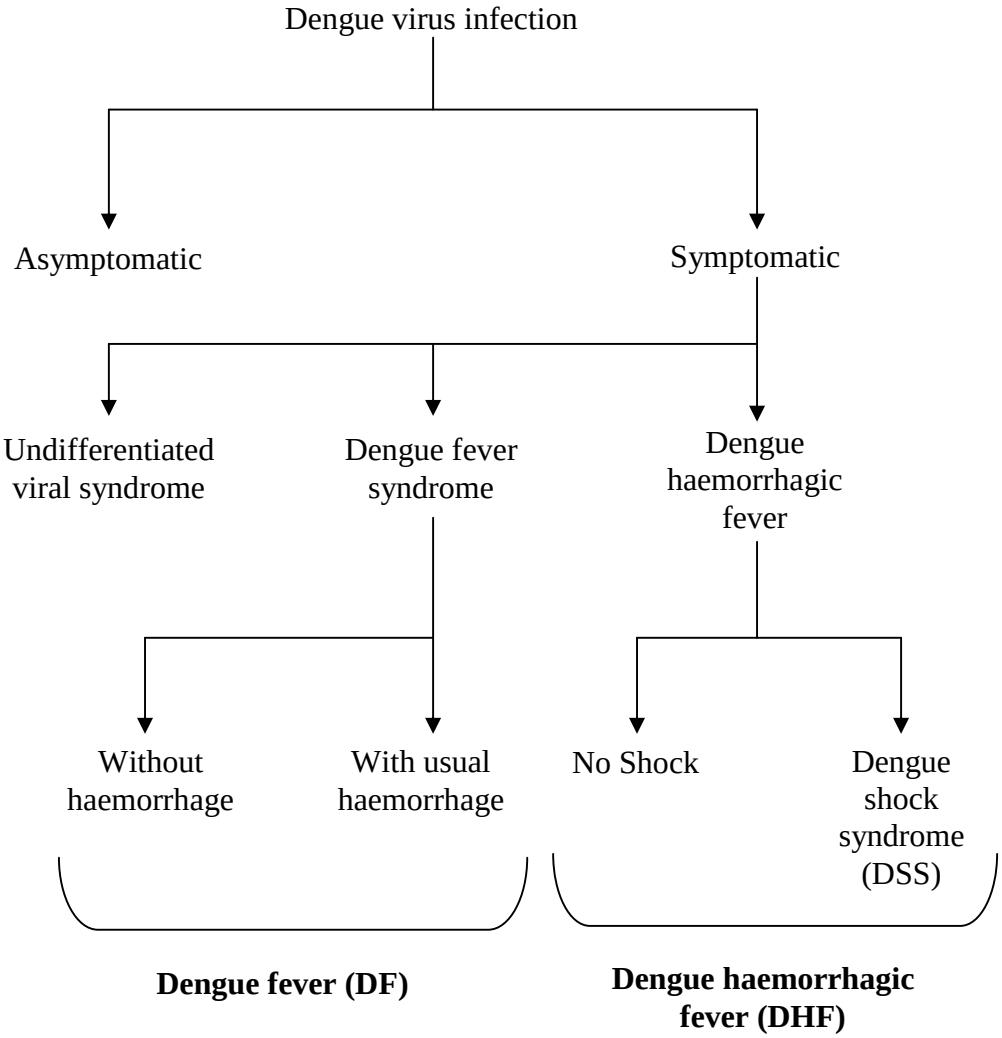
Dengue fever

Dengue fever is an acute viral infection caused by at least one of the four serotypes (1,2,3 and 4) of dengue virus. All ages and both sexes are susceptible to dengue fever. The illness is characterized by an incubation period of three to ten days. The onset is sudden with chills and high fever, intense headache, muscle pain, joint or bony pain (Break bone fever), retro orbital pain and photophobia. Other common symptoms include weakness, abdominal pain, sore throat and general depression.

Fever is usually between 39°C and 40°C, followed by a remission of a few hours to two days (biphasic fever or saddle back fever).

The skin eruptions in 80 percent of case appear during the remission or during second febrile phase, which lasts for one to two days. The rash may be diffuse flushing, mottling or fleeting pinpoint eruptions or the rash may be maculopapular or scarlatiniform.

Figure 3. Spectrum of clinical features of dengue virus infection[35]



Some patients with dengue fever have evidence of mucosal or cutaneous bleeding without other evidence of DHF/DSS like hemoconcentration or fluid leak; such patients are classified as dengue fever with unusual bleeding.[40]

Fever lasts for about five to seven days after which recovery is usually complete although convalescence may be protracted.

Dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS)

DHF/DSS is a severe form of dengue fever, caused by infection with more than one dengue virus and may be fatal in 40-50% of untreated patients. The disease is confined exclusively to children less than 15 years of age, but due to change in epidemiological trend the disease may occur in adult population.

After an incubation period of four to six days the patient develops clinical features of dengue fever. There may be varying degree of tender hepatomegaly or less commonly splenomegaly. All patients have some degree of haemorrhagic phenomenon like positive tourniquet test, petechial spots, bruising at venepuncture site, bleeding from gums, epistaxis, hemetemesis or melena, muscle hematoma, hematuria and rarely intracranial haemorrhage may occur.

Fever may subside after two to seven days. At this stage patient may develop varying degree of peripheral circulatory failure. With progressive peripheral circulatory failure, patient may have sweating, restlessness, cold extremities, pulse pressure gets narrow, blood pressure starts falling ultimately leading to unrecordable blood pressure and irreversible shock.

Unusual manifestations of DHF/DSS include hepatitis, encephalitis and glomerulopnephritis.[40]

Several studies[30,41-51] have reported different clinical features and complications of dengue fever. Thrombocytopenia is a very important indicator of prognosis in DHF as was shown by the study conducted in Philippines by Chua MN, et al.[42] in 1992.

Sharma S et al.[43] from AIIMS, New Delhi studied 98 adult patients diagnosed to have dengue haemorrhagic fever (DHF) (n=75) and dengue shock syndrome (DS) (n=23) during an epidemic of dengue fever in the middle of August 1996. Fever (100%), body aches (45.9%), abdominal pain (38.7%), purpura (33.6%), epistaxis (32.6%), malena (26.5%), haematemesis (22.4%) and ecchymoses (20%) were commonly present symptoms. ELISA IgM antibodies for serodiagnosis of dengue virus infection was positive in 23 of the 27 patients tested. At the time of admission, 94 patients had a platelet count below 100,000/mm3. Four patients with haemorrhagic manifestations had an initial platelet count of >100,000/mm3. Severe thrombocytopenia (platelet count <20,000 /mm^3) was present in 43.8% of the patients. The ultrasound tests showed pleural effusion in 10 of the 12 patients and ascites in five patients tested when they were not clinically evident.

Wali JP et al.[44] studied 17 consecutive patients of DHF/DSS in New Delhi to assess cardiac function by radionuclide ventriculography, echocardiography and electrocardiography (ECG) during the epidemic of Dengue virus type-2 (DENV-2) in Delhi, India (1996). Fourteen patients were seropositive for Dengue infection. In radionuclide ventriculography study, the mean left-ventricular ejection fraction was 41.69 (5.04% (range 33-49%) and 7 patients had an ejection fraction less than 40%, global hypokinesia was detected in 12 (70.59%) patients. In echocardiography, the mean ejection fraction was 47.06 (3.8%). Eight patients had Dengue Shock Syndrome and the mean ejection fraction was 39.63%. Authors concluded that, acute reversible cardiac insult may be noticed in DHF and DSS could be responsible for hypotension/shock.

Kuo CH et al.[45] studied the impact of dengue on liver function by biochemical tests on 125 male and 145 female patients diagnosed with this disease during an outbreak that extended from November 1987 to December 1988 in Taiwan. Abnormal levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, alkaline phosphatase, and gamma-glutamyl transpeptidase (GGT) were observed in 93.3%, 82.2%, 7.2%, 16.3% and 83.0% of the patients, respectively. Study concluded that, dengue fever might cause hepatic injury and transaminase elevation similar to that in patients with conventional viral hepatitis. In epidemic or endemic areas, dengue fever infection should be considered in the differential diagnosis of hepatitis.

Mohan B et al.[46] prospectively studied hepatic functions of 61 children, diagnosed to have dengue infection, aged 2 months to 12 years comprising 37 cases of dengue fever (DF), 16 with dengue hemorrhagic fever (DHF), and eight with dengue shock syndrome (DSS) during the acute attack. Hepatomegaly (74%), epistaxis (26%), jaundice (25%), and petechial rashes (18%) were the common clinical manifestations of dengue infection. On admission, levels of serum aspartate transaminase (AST), serum alanine transaminase (ALT) and serum alkaline phosphatase were raised in 80-87% of children with hepatomegaly (group I) and 81% of cases without hepatomegaly (group II). During the second week of hospitalization the proportion of cases with raised levels of AST, ALT, alkaline phosphatase and serum bilirubin increased and the mean levels were significantly higher ($p<0.05$) in both the groups. Authors suggested that, transient derangement of liver functions in childhood dengue infection, more so in DSS and DHF, with or without hepatomegaly.

In a study by Shivbalan S et al.[47] during 2004 on the predictors of spontaneous bleeding in dengue, a platelet count of less than 50,000 was found to be significantly associated with increased risk of bleeding. The other associated predictors of bleeding in the study conducted were prolonged PT, raised AST/ALT and haemoconcentration.

Shah I et al.[48] conducted prospective study in the pediatric wards and pediatric intensive care unit of B. J. Wadia Hospital, Mumbai for Children between 27 August 2003 and 10 October 2003 to determine the clinical features of children affected with dengue. Fever, hepatomegaly, vomiting, bleeding tendencies, erythematous rash, thrombocytopenia, elevated liver enzymes, and deranged PT and PTT were the predominant clinical and laboratory features. Predictive markers for DSS were younger age at onset, altered sensorium, paralytic ileus, and significantly deranged PT.

In a study by Venkat Sai PM et al.[49] on the role of USG in dengue fever, 100% of the patients showed gall bladder thickening and pericholecystic fluid, 21% had hepatomegaly, 6.25% had spleenomegaly and minimal right pleural effusion. In a follow up USG on the 5th day in the same patients, 53% had ascites. Study concluded that, in an epidemic of dengue, ultrasound features of thickened gall bladder wall, pleural effusion and ascites should strongly favour the diagnosis of dengue fever.

Recently, Kumar A et al.[50] in his record-based study conducted in a coastal district of Karnataka to study the clinical manifestations, trend and outcome of all confirmed dengue cases admitted in a tertiary care hospital

assessed the laboratory confirmed cases from 2002 to 2008 from Medical Records Department (MRD). Of the 466 patients, the most common presentation was fever 462 (99.1%), followed by myalgia 301 (64.6%), vomiting 222 (47.6%), headache 222 (47.6%) and abdominal pain 175 (37.6%). The most common hemorrhagic manifestation was petechiae (67.2%). Of the 66 (14.1%) patients who developed clinical complications, 22 (33.3%) had ARDS and 20 (30.3%) had pleural effusion.

More recently Karoli R et al.[51] in their cross-sectional study at Lucknow during the monsoon and post-monsoon seasons in the year 2010 on 356 patients with suspected dengue fever found 138 (39%) had serologically confirmed dengue infection. Out of this Ninety-six (70%) patients had classical dengue fever while 42 (30%) had dengue hemorrhagic fever. The most common symptoms were headache (105, 76%), abdominal pain (87, 63%), vomiting (80, 58%), rash (36, 26%) and cutaneous hypersensitivity (22, 16%). Hemorrhagic manifestations were present in 55 (40%) patients. Notably, 14% of patients had neurological involvement and 4% had acute hepatic failure. Study concluded that, dengue infection had varied and multi-systemic manifestations that can go unrecognized.

Also there is high index of suspicion of the various atypical clinical presentations involving various organs / systems which include; [30]

- Neurological manifestations - Encephalopathy, acute motor weakness, seizures, neuritis, Guillain Barre syndrome, hypokalemic paralysis acute viral myositis, acute encephalitis;

- Hepatic involvement - Acute liver failure, hepatic encephalopathy, hepatomegaly, jaundice and petechial rashes;
- Myositis - Acute myositis, pure motor quadriplegia;
- Cardiac involvement - Acute reversible cardiac insult, sinoatrial block and atrioventricular dissociation;
- Lupus erythematosus (systemic) - Abnormal immune response leading to systemic lupus erythematosus;
- Occlar complications & uveitis - Unilateral blurring of inferior visual field;
- Acute renal dysfunction - Renal dysfunction, acute kidney injury; Acute inflammatory colitis such as Lower gastrointestinal bleeding and acute inflammatory colitis;
- Cutaneous manifestations - Maculopapular/morbilliform eruption followed by ecchymotic, petechial, and macular/scarlatiniform eruption, Confluent erythema, morbilliform eruptions, and haemorrhagic lesions;
- Kawasaki disease - Young child developed Kawasaki disease later in disease and Bone marrow haemophagocytosis associated with nasal bleeding and pancytopenia

Laboratory diagnosis

Dengue virus infections can result in a range of clinical manifestations from asymptomatic infection to dengue fever (DF) and the severe disease, dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS). Most dengue infections are asymptomatic or cause mild symptoms, which are characterized by

undifferentiated fever with or without rash. Typical DF is characterized by high fever, severe headache, myalgia, arthralgia, retro-orbital pain and maculopapular rash. Some patients show petechiae, bruising or thrombocytopenia. The clinical presentation of acute dengue infection is non-specific but 5–10% of patients progress to severe DHF/DSS, which can result in death if it is not managed appropriately. Plasma extravasation is the main pathophysiological finding of DHF/DSS, which differentiates it from DF. DHF/DSS is characterized by high fever, bleeding, thrombocytopenia and haemoconcentration (an increase in the concentration of blood cells as a result of fluid loss). Approximately 3–4 days after the onset of fever, patients can present with petechiae, rash, epistaxis, and gingival and gastrointestinal bleeding. Pleural effusion and ascites are common. Some patients develop circulatory failure (DSS), presenting with a weak and fast pulse, narrowing of pulse pressure or hypotension, cold and moist skin and altered mental state. Although there are no specific antiviral treatments for dengue infection, patients usually recover when the need for fluid management is identified early and electrolytes are administered.[52]

There is a need for specific, inexpensive dengue diagnostic tests that can be used for clinical management, surveillance and outbreak investigations and would permit early intervention to treat patients and prevent or control epidemics. Progress is being made in primary prevention, with several candidate dengue vaccines in late phases of development as well as improved vector control measures. Additionally, new techniques for the early detection of severe disease such as the use of biomarkers have the potential to decrease morbidity and mortality. Recent developments in rapid detection technologies offer the promise

of improved diagnostics for case management and the early detection of dengue outbreaks.[52]

The characteristics of an 'ideal' dengue diagnostic test depend on the purpose for which the test will be used. The optimal window for diagnosing a dengue infection is roughly from the onset of fever to 10 days post-infection; however, as not all patients are diagnosed within this period, an ideal diagnostic test should be sensitive regardless of the stage of infection. Laboratory confirmation of dengue infection relies on isolation of the virus in cell culture, the identification of viral nucleic acid or antigens, or the detection of virus-specific antibodies.[53]

Direct virus detection could potentially be used for early, definitive and serotype-specific identification of dengue infections during the acute phase of the disease. Live virus or virus components (RNA or antigens) can be detected in serum, plasma, whole blood and infected tissues from 0–7 days following the onset of symptoms, which corresponds roughly to the duration of fever. Laboratories do not routinely perform direct virus detection procedures, and few commercial kits that have been independently validated are available to aid in this area of dengue diagnosis.[52]

Serological tests are more commonly used to diagnose dengue infections because of their ease of use compared to techniques such as cell culture or RNA detection. Different patterns of antibody responses are observed when patients experience a first (primary) or subsequent (secondary) dengue infection. In primary infections, immunoglobulin M (IgM) is detected 5 or more days after the

onset of illness in the majority of infected individuals and immunoglobulin G (IgG) is detected from 10–15 days. In secondary infections, IgM appears earlier or in the same time frame but are usually at lower titres than in primary infection. IgG is present from the previous infection and the titre increases rapidly. Haemagglutination inhibition (HAI) antibody titres in primary infections peak at 1:640 whereas titres of 1:1280 or greater are common in secondary infections.[53]

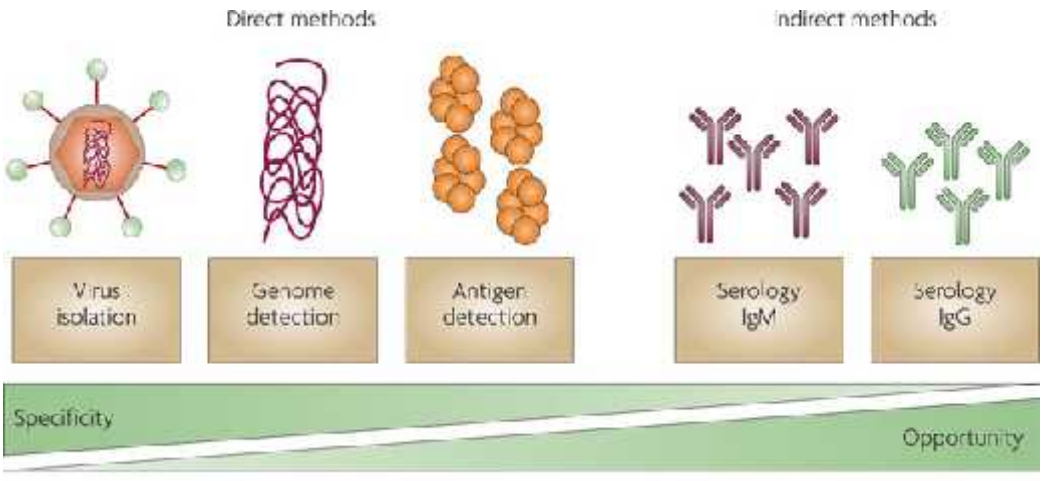


Figure 4. Comparative merits of direct and indirect laboratory methods for the diagnosis of dengue infections[52]

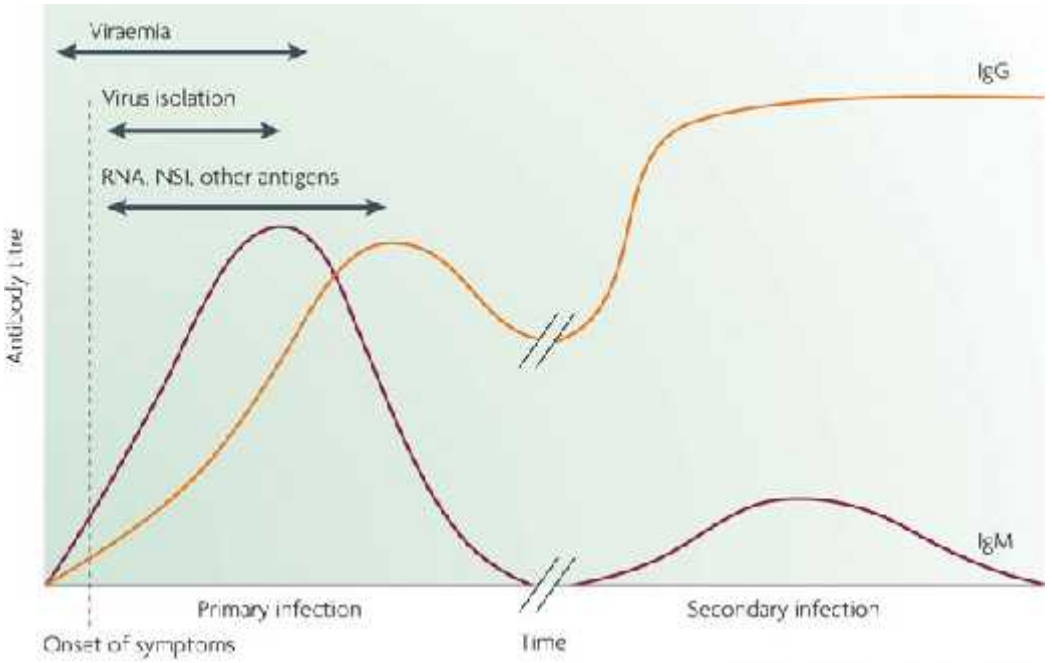


Figure 5. Major diagnostic markers for dengue infection[52]

Several rapid diagnostic tests are commercially available and many in-house assays have been developed but the performance characteristics of many of these tests have not been adequately evaluated. The International Expert Meetings on dengue diagnostics held at the WHO in Geneva in October 2004 and April 2005 recommended the need for a validated diagnostic test for dengue virus infection for clinical and epidemiological use. The purpose of this guide is to establish best practice guidelines for how to design and conduct evaluations of dengue diagnostic tests for the management of acute infections, surveillance and monitoring of interventions.[52]

Current laboratory methods for dengue diagnosis

The tests that are currently used in the laboratory diagnosis of dengue infections, and their advantages and limitations, are shown in Table 1.

Advantages and limitations of different dengue diagnostic tests[52]

Diagnostic tests	Advantages	Limitations
Viral isolation and identification	• Confirmed infection • Specific • Identifies serotypes	• Requires acute sample (0–5 days post onset) • Requires expertise and appropriate facilities • Takes more than 1 week • Does not differentiate between primary and secondary infection • Expensive
RNA detection	• Confirmed infection • Sensitive and specific • Identifies serotype and genotype • Results in 24–48 hours	• Potential false-positives owing to contamination • Requires acute sample (0–5 days post onset) • Requires expertise and expensive laboratory equipment • Does not differentiate between primary and secondary infection
Antigen detection		
Clinical specimens (for example, using blood in an NS1 assay)	• Confirmed infection • Easy to perform • Less expensive than virus isolation or RNA detection	• Not as sensitive as virus isolation or RNA detection
Tissues from fatal cases (for immuno-histochemistry, for example)	• Confirmed infection	• Not as sensitive as virus isolation or RNA detection • Requires expertise in pathology
Serological tests		
IgM or IgG seroconversion	• Confirmed infection • Least expensive • Easy to perform	• IgM levels can be low in secondary infections • Confirmation requires two or more serum samples • Can differentiate between primary and secondary infection*
IgM detection (single sample)	• Identifies probable dengue cases • Useful for surveillance, tracking outbreaks and monitoring effectiveness of interventions	• IgM levels can be low in secondary infections

*Primary infection: IgM-positive and IgG-negative (if samples are taken before day 8–10); secondary infection: IgG should be higher than 1,280 haemagglutination inhibition in convalescent serum.

Virus detection

Dengue virus can be isolated by the inoculation of diagnostic samples into mosquitoes, cell culture (using mosquito cell lines, such as C6/36 and AP61 or mammalian cell lines, such as Vero and LLC-MK2 cells) or intra-cerebral inoculation of suckling mice. Whole blood, serum or plasma is collected from patients during the acute phase of the disease or from tissues in fatal cases (dengue virus has also been isolated from liver, spleen, lymph nodes and other tissues). There is evidence that the virus isolation rates from whole blood are considerably higher than the isolation rates from serum or plasma.[54] For virus serotype identification, immunofluorescent assays using serotype-specific monoclonal antibodies (mAbs) are used.[52]

Viral RNA detection

Dengue viral RNA can be detected using a nucleic acid amplification test (NAAT) on tissues, whole blood or sera taken from patients in the acute phase of the disease. Various protocols have been developed that identify dengue viruses using primers directed to serotype-specific regions of the genome.[55-57] Nested PCR techniques improve the sensitivity of detection because the initial amplification product is used as the target for a second round of amplification. However, it is crucial that laboratories performing nested PCR take every precaution to prevent false-positive results that can occur as a result of contamination. In situ PCR can be carried out on tissue slides.[52]

Antigen detection

NS1-based assays

A simplified method of diagnosis of dengue infection during the acute stage compared to viral isolation or nucleic acid detection is the detection of viral antigens in the bloodstream. However, antigen detection in the acute stage of secondary infections can be compromised by pre-existing virus–IgG immunocomplexes.[52]

New developments in enzyme-linked immunosorbent assay (ELISA) and rapid immunochromographic assays that target non-structural protein 1 (NS1) have shown that high concentrations of this antigen can be detected in patients with primary and secondary dengue infections up to 9 days after the onset of illness. NS1 is synthesized by all flaviviruses and is secreted from infected mammalian cells. The presence of secreted NS1 (sNS1) in the bloodstream stimulates a strong humoral response. Many studies have investigated the utility of sNS1 detection as a diagnostic tool during the acute phase of a dengue infection. A serotype specific monoclonal antibody (mAb) based NS1 antigen-capture ELISA has recently been developed and shows good serotype specificity. This test can differentiate between primary and secondary dengue virus infections. There is a good correlation between NS1 serotype-specific IgG as determined by ELISA and plaque reduction neutralization test (PRNT) results, but the performance and utility of these NS1-based tests require additional evaluation.[52]

Immunohistochemistry

Dengue antigens can be visualized in tissue sections using labeled mAbs that are visualized using markers like fluorescent dyes, enzymes or colloidal gold. These tests can be undertaken on frozen tissue or paraffin-embedded slides from fatal cases.

Serological methods

The acquired immune response to dengue virus infection consists of the production of immunoglobulins (IgM, IgG and IgA) that are mainly specific for the virus envelope (E) protein. The intensity of the response varies depending on whether the individual has a primary or secondary dengue infection. During a primary dengue infection, the IgM has a typically higher titre and is more specific than during secondary infections. The titre of the IgG response is higher during secondary infection than during primary infection (Figure 5). IgA and IgE-based assays have also been used but the utility of these immunoglobulins as markers for the serodiagnosis of dengue infections requires further validation.

It is difficult, if not impossible; to use serology to identify dengue serotypes following a recent infection because the antibodies produced following a primary dengue infection often demonstrate some degree of cross-reactivity with other dengue virus serotypes. Antibodies formed following secondary dengue infections are strongly cross-reactive within the dengue group and also usually cross-react with other flaviviruses.[58]

IgM-based assays

The detection of dengue-specific IgM is a useful diagnostic and surveillance tool. IgM is initially detectable between 3 to 5 days post onset of fever in ~50% of hospitalized patients and has a sensitivity and specificity of ~90% and 98%, respectively, when assays are undertaken five days or more after the onset of fever. Dengue-specific IgM is expressed earlier than dengue-specific IgG. In one study in Puerto Rico, by day 5 of illness, most patients (80%) with dengue infections that were subsequently confirmed by HAI on paired serum samples or by virus isolation had detectable IgM in acute-phase serum. Nearly all patients (93%) developed detectable IgM 6 to 10 days after the onset of fever, and 99% of patients tested between 10 and 20 days after onset had detectable IgM.[59]

The sensitivity and specificity of IgM-based assays is strongly influenced by the quality of the antigen used and can vary greatly between commercially available products. ELISA-based IgM assays have become an invaluable tool for the surveillance of dengue. Many ELISAs use dengue E protein antigens from all four dengue virus serotypes. This ensures that the assay is capable of identifying any dengue infection regardless of the serotype. However, because IgM circulates for up to three months or longer, its presence might not be diagnostic of a current illness. To diagnose a current dengue infection, the demonstration of a seroconversion (four fold or greater changes in antibody titres) in paired sera is required.[52]

In areas where dengue is not endemic, IgM-based assays can be used in clinical surveillance for viral illness or for random, population-based serological surveys, with the likelihood that any positive results detected indicate recent infections (within the past 2 to 3 months). The IgM antibody capture ELISA (MAC-ELISA) is based on detecting IgM in serum using human-specific IgM that is bound to the solid phase. MAC ELISAs are frequently run as a non-quantitative, single dilution test and positive results are commonly reported as a 'recent flavivirus infection. [52]

Rapid IgM-based dengue diagnostic tests have been developed as a quick and easy method for use at point of care or bedside, and exist in different formats including particle agglutination and lateral flow immunochromatographic strips, with or without plastic cassettes. Most of these tests use recombinant antigens from all four dengue virus serotypes and the results are available within 15 to 90 minutes. Four rapid IgM-based kits have been evaluated. Their sensitivities ranged from 21%–99% and their specificities from 77%–98%, compared with gold standard laboratory-based ELISAs. False-positive results were present in patients with malaria or previous dengue infections.[3, 60] The ELISA format shows greater sensitivity in detecting dengue-specific antibodies than the rapid tests, but the rapid tests are field friendly, with the results available in a short timeframe.

IgG-based assays

Dengue-specific IgG-based assays can be used for the detection of past dengue infections and current infections if paired sera are collected within the correct time frame to allow the demonstration of seroconversion between acute

and convalescent serum samples. Assays are usually carried out using multiple dilutions of each serum tested to determine an end-point titre.[52]

IgG avidity assays can be used to determine whether an infection is a primary or a secondary infection, based on the principle that the avidity of IgG is low after primary antigenic challenge but matures slowly within the weeks and months after infection. Thus, these assays can be more useful than the HAI test for this purpose. The IgG-based ELISA exhibits the same broad cross-reactivity with other flaviviruses as the HAI test; therefore, it cannot be used to identify the infecting dengue virus serotype. However, it has a slightly higher sensitivity than the HAI test.[52]

Role of NS1 antigen detection

The most important development in dengue diagnostics in recent years is the advent of the specific detection of dengue virus NS1 antigen. Dengue RDTs that detect NS1 antigen employ a number of serotype-specific anti-NS1 monoclonal antibodies to capture and detect soluble NS1 antigen in serum, plasma, or blood. The first commercial assays for dengue NS1 antigen detection used the ELISA format[61,62] and demonstrated excellent sensitivity and specificity in the early phase of infection that diminished with falling viraemia levels. The major commercial diagnostics manufacturers, Panbio, Biorad, and SD, have all developed RDT-based NS1 antigen tests, and have equivalent ELISA-based assays.[62]

Twelve studies evaluated the Biorad STRIP RDT for the diagnosis of acute dengue infection using admission samples, and the results demonstrated

considerable variation in sensitivity (49.8%–98.7%) but the specificities reported were more consistent with all being >90%. For 25% (3/12) of the studies, the sensitivity was >89%; however, all of these studies used a skewed comparator of either virus isolation, RT-PCR, or NS1-ELISA and did not examine the possibility of false-negative results by testing paired serum samples to examine for dynamic rise in serological assays such as IgM (MAC) or IgG (GAC) capture ELISAs.[62]

Studies that used a more representative combination of virus or antigen detection and serology as reference comparators gave sensitivities for the Biorad STRIP RDT of between 49.4%[63] and 78.9%. [64]

The SD Bioline Dengue Duo RDT NS1 antigen detection strip was evaluated for acute dengue diagnosis in four studies with consistently high specificity estimates (96.7–100%) and sensitivities that ranged from 48.5%[65] to 65.4%[66] with the studies either using a combination of virus detection and serology[63-64] as comparators or serology alone.[65]

The Panbio Early Rapid RDT NS1 antigen detection strip was evaluated in two studies using samples from three locations (Vietnam, Malaysia, and Sri Lanka) with high specificity estimates (92.5–96.7%) and sensitivities that ranged from 58.6%[65] to 69.2%[66] for admission samples.

A few studies have compared the diagnostic accuracy of NS1 antigen RDTs in primary and secondary dengue infections. Generally, NS1-antigen RDTs demonstrated higher sensitivities in primary infections when compared to secondary infections;[67-70] however, other studies have reported the opposite.[71]

It has been suggested that this phenomenon of lowered NS1-antigen detection in dengue secondary infections is caused by NS1 antigen complexing with anti-NS1 antibodies.[63] This observation results in an inability of the NS1-antigen RDT to detect complexed NS1 antigen and should not be interpreted insensitivity on the part of the diagnostic assay.[62]

To take advantage of the entire temporal spectrum of patient presentation during the acute phase of dengue infection (usually from 1 to 7 days after onset of fever), NS1 antigen and IgM antibody results have been combined in a Boolean manner using AND/OR operators. NS1 antigen is present in the serum in the early phase of infection; however, patients that present late in the course of infection may have undetectable levels of NS1 antigen. Dengue IgM antibodies are usually present following 2–5 days of infection, and, by combining the results of dengue NS1 antigen and IgM antibody testing, accurate diagnosis during acute presentation is possible. This approach was initially described by combining the results of the Panbio NS1 antigen and IgM antibody ELISAs.[62]

Subsequently, studies[65-66] have combined NS1 antigen and antibody results to exploit the temporal diagnostic characteristics of each analyte. Combining the SD Bioline Dengue Duo RDT NS1 antigen and IgM antibody results for acute diagnosis, the sensitivity ranged from 75.5%[67] to 92.9%[65] and the specificity from 88.8%[65] to 100%.[67] Combining the Panbio Early Rapid RDT NS1 antigen and IgM antibody results, the sensitivity ranged from 89.0% to 89.9%; and the specificity reported was 75.0%.[65]

Other markers

The following laboratory tests should also be performed:

- Complete blood count (CBC)
- Metabolic panel
- Serum protein and albumin levels
- Liver function tests
- Disseminated intravascular coagulation (DIC) profile
- Renal function test

Characteristic findings in dengue fever are thrombocytopenia (platelet count < 100 x 10^9/L), leukopenia, and mild-to-moderate elevation of aspartate aminotransferase and alanine aminotransferase values. Jaundice and acute liver failure are uncommon. Peak liver enzyme levels occur later than other complications in adults as studied prospectively in Vietnam. Enzyme levels begin to rise during the early stage and peak during the second week. Clinically severe involvement was found to be idiosyncratic and infrequent but did contribute to severe bleeding.[72]

A hematocrit level increase greater than 20% is a sign of hemoconcentration and precedes shock. The hematocrit level should be monitored at least every 24 hours to facilitate early recognition of dengue hemorrhagic fever and every 3-4 hours in severe cases of dengue hemorrhagic fever or dengue shock syndrome.

In patients with dengue hemorrhagic fever, the following may be present:

- Increased hematocrit level secondary to plasma extravasation and/or third-space fluid loss.
- Hypoproteinemia.
- Prolonged prothrombin time.
- Prolonged activated partial thromboplastin time.
- Decreased fibrinogen.
- Increased amount of fibrin split products

Signs of early coagulopathy may be as subtle as a guaiac test that is positive for occult blood in the stool. Guaiac testing should be performed on all patients in whom dengue virus infection is suspected.

Typing and crossmatching of blood should be performed in cases of severe dengue hemorrhagic fever or dengue shock syndrome because blood products may be required.

Urinalysis identifies hematuria. Cultures of blood, urine, CSF, and other body fluids should be performed as necessary to exclude or confirm other differential diagnosis.

Arterial blood gas should be assessed in patients with severe cases to assess pH, oxygenation, and ventilation.

Electrocardiography may demonstrate nonspecific changes as a result of fever, electrolyte disturbances, tachycardia, or medications. The usefulness of these changes as a marker of cardiac involvement is unclear.

Biopsy of the skin lesions in patients with nonfatal, uncomplicated dengue fever reveals an abnormality of the small blood vessels. Endothelial swelling, perivascular edema, and mononuclear cell infiltration are the primary histologic findings.

Perform chest radiography to look for pleural effusions and bronchopneumonia. Right-sided pleural effusion is typical. Bilateral pleural effusions are common in patients with dengue shock syndrome. Head computed tomography without contrast may be indicated in patients with altered level of consciousness, to detect intracranial bleeding or cerebral edema from dengue hemorrhagic fever.

Complete Blood Cell Count

Leukopenia, often with lymphopenia, is observed near the end of the febrile phase of illness. Lymphocytosis, with atypical lymphocytes, commonly develops before defervescence or shock. A systematic review found that patients with dengue had significantly lower total WBC, neutrophil, and platelet counts than patients with other febrile illnesses in dengue-endemic populations.[73]

A hematocrit level increase greater than 20% is a sign of hemoconcentration and precedes shock. The hematocrit level should be monitored at least every 24 hours to facilitate early recognition of dengue hemorrhagic fever and every 3-4 hours in severe cases of dengue hemorrhagic fever or dengue shock syndrome.

Thrombocytopenia has been demonstrated in up to 50% of dengue fever cases. Platelet counts less than 100,000 cells/μL are seen in dengue hemorrhagic fever or dengue shock syndrome and occur before defervescence and the onset of shock. The platelet count should be monitored at least every 24 hours to facilitate early recognition of dengue hemorrhagic fever.

Metabolic Panel and Liver Enzymes

Hyponatremia is the most common electrolyte abnormality in patients with dengue hemorrhagic fever or dengue shock syndrome. Metabolic acidosis is observed in those with shock and must be corrected rapidly. Elevated blood urea nitrogen (BUN) levels are observed in those with shock. Acute kidney injury is uncommon.[74,75]

Transaminase levels may be mildly elevated into the several thousands in patients with dengue hemorrhagic fever who have acute hepatitis. Low albumin levels are a sign of hemoconcentration.

Coagulation Studies

Coagulation studies may help to guide therapy in patients with severe hemorrhagic manifestations. Findings are as follows:

- Prothrombin time is prolonged.
- Activated partial thromboplastin time is prolonged.
- Low fibrinogen and elevated fibrin degradation product levels are signs of disseminated intravascular coagulation

Ultrasonography

Ultrasonography is a potentially timely, cost-effective, and easily used modality in the evaluation of potential dengue hemorrhagic fever. Positive and reliable ultrasonographic findings include fluid in the chest and abdominal cavities, pericardial effusion, and a thickened gallbladder wall. Thickening of the gallbladder wall may presage clinically significant vascular permeability.[76]

The utility of previous studies was limited because patients underwent only a single scan. However, in a study by Srikiatkhachorn et al, daily serial ultrasonographic examinations of the thorax and abdomen proved useful in the evaluation of patients with suspected dengue hemorrhagic fever.[76]

Plasma leakage was detected in some patients within 3 days of fever onset. Pleural effusion was the most common sign. Based on ultrasonographic findings, dengue hemorrhagic fever was predicted in 12 patients before hemoconcentration criteria had been met.

Case Definitions

Cases are classified as probable dengue if they are compatible with the clinical definition and satisfy one or more of the following criteria:

- Supportive serology (reciprocal hemagglutination inhibition antibody titer greater than 1280, comparable IgG EIA titers, or positive IgM antibody test in late acute or convalescent-phase serum specimen).
- Occurrence at the same location and time as other confirmed cases of dengue fever.

- A confirmed case of dengue is one that is compatible with the clinical definition and is confirmed by the laboratory.

Case definition

WHO 1997 case definitions for DF, DHF and DSS[77,78]

Dengue Fever

Probable

- An acute febrile illness with two or more of the following manifestations: headache, retro-orbital pain, myalgia, arthralgia, rash, haemorrhagic manifestations and leucopenia

  And

- Supportive serology (a reciprocal haemagglutination-inhibition antibody titre ≥1280, a comparable IgG enzyme-linked immunosorbent assay (ELISA) titre or a positive IgM antibody test on a late acute or convalescent-phase serum specimen).

  Or

- Occurrence at the same location and time as other DF cases

Confirmed

- A case confirmed by one of the following laboratory criteria:
- Isolation of the dengue virus from serum/autopsy samples
- At least four-fold change in reciprocal IgG/IgM titres to one or more dengue virus antigens in paired samples

- Demonstration of dengue virus antigen in autopsy tissue, serum or cerebrospinal fluid samples by immunohistochemistry, immunofluorescence or ELISA
- Detection of dengue virus genomic sequences in autopsy tissue serum or cerebrospinal fluid samples by polymerase chain reaction (PCR)

Reportable

- Any probable or confirmed case should be reported

Dengue haemorrhagic fever

For a diagnosis of DHF, a case must meet all four of the following criteria:

- Fever or history of fever lasting 2–7 days, occasionally biphasic
- A haemorrhagic tendency shown by at least one of the following: a positive tourniquet test; petechiae, ecchymoses or purpura; bleeding from the mucosa, gastro-intestinal tract, injection sites or other locations; or haematemesis or malena
- Thrombocytopenia [≤ 100,000 cells/mm^3 (100×10^9/L)]
- Evidence of plasma leakage owing to increased vascular permeability shown by: an increase in haematocrit ≥20% above the average for age, sex and population; a decrease in the haematocrit after intervention ≥ 20% of baseline; signs of plasma leakage such as pleural effusion, ascites or hypoproteinaemia

Dengue shock syndrome

For a case of DSS, all four criteria for DHF must be met, in addition to evidence of circulatory failure manifested by:

- Rapid and weak pulse *and* narrow pulse pressure (<20 mmHg or 2.7 kPa)

  Or

- *manifested by* Hypotension for age a*nd* Cold, clammy skin and restlessness

The 2009 WHO criteria classify dengue according to levels of severity: dengue without warning signs; dengue with warning signs (abdominal pain, persistent vomiting, fluid accumulation, mucosal bleeding, lethargy, liver enlargement, increasing haematocrit with decreasing platelets); and severe dengue (dengue with severe plasma leakage, severe bleeding, or organ failure). [79]

Patients who recover following defervescence are considered to have non-severe dengue, but those who deteriorate tend to manifest warning signs.[6] These individuals are likely to recover with intravenous rehydration. However, further deterioration is classified as severe dengue, though recovery is possible if appropriate and timely treatment is given.[79]

Figure 6. The 2009 revised dengue case classification[79]

The 2009 classification into severity levels is considered to be more sensitive in capturing severe disease than the 1997 guidelines, with observed sensitivities of up to 92% and 39%, respectively. [80,81]

A multi-centre study across 18 countries demonstrated that approximately 14% of cases could not be classified using the DF/DHF/DSS classification, even when strict DHF criteria were not applied, compared with only 1.6% with the revised system.[77] The study also examined the acceptance and user-friendliness to healthcare professionals.[77] The new classification was particularly useful with respect to triage and management of dengue, reporting during surveillance and for endpoint measurements in dengue clinical trials.[77,79]

Comparison of the 1997 and revised classifications[77]

DF/DHF/DSS classification by expert reviewer	Revised classification by expert reviewer				Total
	Not classifiable*	Dengue WS negative	WS positive	Severe dengue	
Not classifiable	23 (8·6%)	57 (21·3%)	159 (59·3%)	29 (10·8%)	268 (100%) (13·7% of all)
DF	7 (0·5%)	551 (41·8%)	684 (51·9%)	75 (5·7%)	1317 (100%) (67·1% of all)
DHF (grades 1 & 2)	2 (0·7%)	8 (2·8%)	240 (83·0%)	39 (13·5%)	289 (100%) (14·7% of all)
DSS (DHF grades 3 & 4)	0	0	12 (13·6%)	76 (86·4%)	88 (100%) (4·5% of all)
Total	32 (1·6%)	616 (31·4%)	1095 (55·8%)	219 (11·2%)	1962 (100%)

The 1997 WHO case classification system for dengue was revised because of differences across the broad geographical areas and the age groups affected by dengue. However, the current 2009 WHO classification is yet to be

proved effective. The question remains, therefore, whether this latest classification requires further modification. A solution may be to incorporate elements from the 2009 classification of severe dengue into the 1997 guidelines, much of which remains relevant for use. This may be resolved by conducting multi-centre, prospective studies using standardized protocols in Asia and Latin America in a full range of patient age groups.[78]

However, problems with the use of the revised classification have also been noted. Additional training for healthcare workers and dissemination of information may be required to remedy any confusion over the changes in the system.[77]

Management

General principles

As there is no specific antiviral treatment, management is essentially supportive and symptomatic. Key to the success is frequent monitoring and strategy changes depending on clinical and laboratory evaluation. As there is plasma leakage in DHF/DSS, intravenous fluid therapy, in the form of crystalloid and colloid therapy.

As the plasma leakage is not constant in rate, the volume and rate of fluid therapy should be adjusted accordingly. However in spite of the massive plasma loss; judicious fluid replacement is necessary to avoid overhydration.

Indications of hospitalization[82]

- Restlessness or lethargy
- Cold extremities or circumoral cyanosis
- Bleeding in any form
- Oliguria or reluctance to take fluid orally
- Rapid and weak pulse
- Capillary refill time > 2 seconds
- Narrowing of pulse pressure (<20mm of Hg) or hypotension)
- Hematocrit of 40 or rising hematocrit
- Platelet count of less than 1,00,000/mm^3
- Acute abdominal pain
- Evidence of plasma leakage eg. Pleural effusion, ascites.

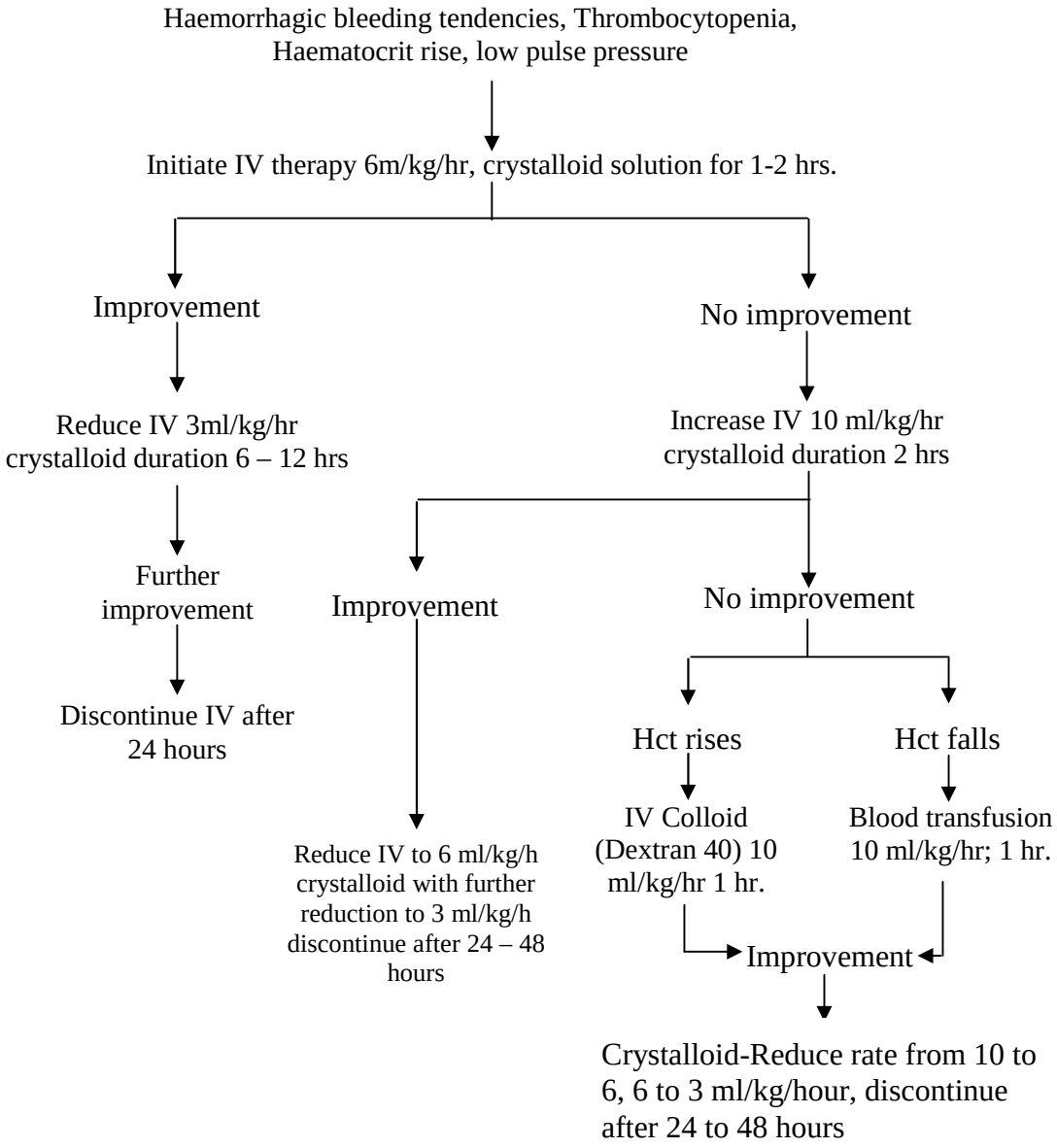
Dengue fever

Patient with dengue fever require rest, oral fluids to compensate for losses via diarrhea or vomiting, analgesics and antipyretics preferably paracetamol. Antibiotics are not indicated in uncomplicated patients.[83]

Dengue hemorrhagic fever Grade I and Grade II

In DHF Grade–I and Grade–II, administered intravenous fluid in the form of isotonic fluid like N.S or R. L at 6 – 7 ml/kg/hr for an hour. After one hour if Hct has decreased and vital parameter are improving fluid infusion rate should be decreased step wise to 3ml kg/hr and maintained for 24-48 hours. If Hematocrit is rising and vitals are deteriorating, stepwise increase in intravenous fluid should be made.

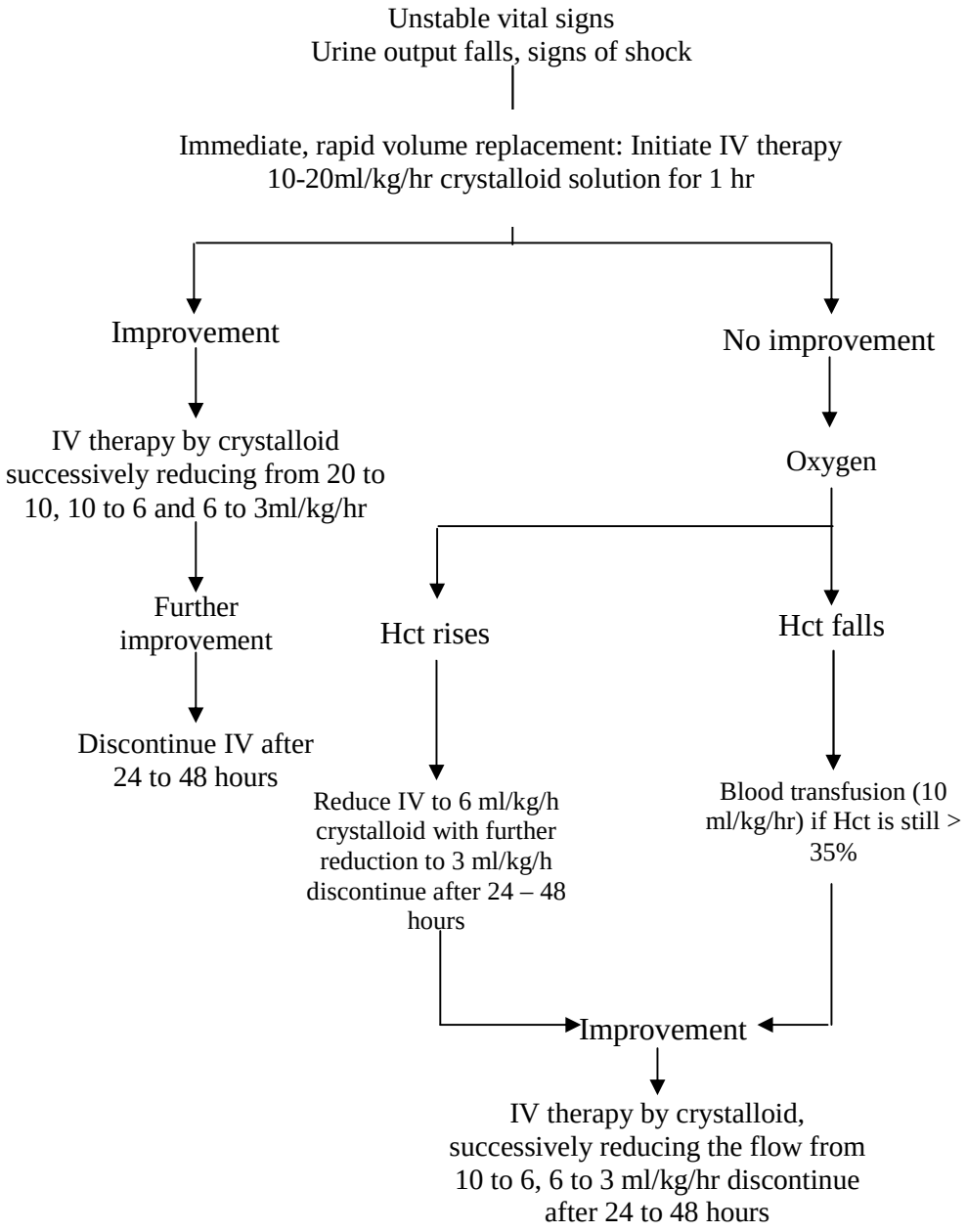
Figure 7. Flow chart of the treatment for DHF Grade – I and Grade – II[36]



DHF Grade – III and Grade – IV[84]

This is life threatening situation in which rapid and massive plasma loss occurring through increased capillary permeability leading to hypotension or shock, for what is required is prompt and adequate fluid replacement with crystalloids or colloids (plasma expanders).

Figure 8. Flow chart of the treatment for DSS[36]



Delayed or inadequate fluid resuscitation can cause multisystem organ dysfunction that may lead to death. Electrolyte and acid base disturbance may occur. There is a high potential for developing disseminated intravascular coagulopathy (DIC) in cases with prolonged shock.

Immediate Replacement of Plasma Loss

Fluid used for rapid volume expansion includes physiological saline or Ringers lactate or Ringer's acetate. N.S or R.L. should be given at 10-20 ml per kg body weight boluses as rapidly as possible, repeat boluses 2-3 times until vital signs return to normal. Oxygen should be given to all patients in shock. If vitals are improving change fluids to 0.45% dextrose saline at rate of 3-6 ml/kg body weight.

If hematocrit is still high and if there is no clinical improvement, plasma substitutes or 5% albumin (10-20 ml/kg body weight) should be given, repeated if necessary for a total dose of 20-30ml/kg body weight of colloidal solution.

If shock still persists, hematocrit values should be revived for any evidence of a decline, which may indicate internal bleeding. Fresh whole blood transfusion (10ml/kg) may be necessary in such cases.

Continued Replacement of Further Plasma Loss

Plasma loss may continue for 24-48 hours requiring continued fluid administration with 5% dextrose in 0.45% normal saline. Decrease in infusion should be done stepwise and in general, intravenous fluid therapy is not needed for more than 48 hours after termination of shock.

Replacement of extravasted plasma and hypervolemia, pulmonary oedema or heart failure may occur if more fluid is given during the recovery phase. At this stage, drop in haematocrit should not be interpreted as a sign of internal bleeding. Strong pulse and blood pressure and adequate diuresis are good signs of recovery.

Use of Blood and Blood Products

Fresh Whole Blood

A drop in hematocrit with no clinical improvement despite adequate fluid administration indicates significant internal hemorrhage. Transfusion with fresh whole blood is preferable and the amount to be given such as normal red blood cell concentration should not be exceeded.

Fresh Frozen Plasma

It is indicated in cases where consumptive coagulopathy causes massive bleeding. DIC is usual in severe shock and may play an important part in the development of massive bleeding or lethal shock.

Platelet Transfusion

It is surrounded with controversies in DHF/DSS. Mild thrombocytopenia usually not associated with significant bleeding. Secondly, thrombocytopenia in DHF/DSS is a short lived phenomenon with platelets returning to normal by 7-9 days.

Platelet transfusion is indicated in adults when platelet count is less than 20,000/mm^3 and have severe hemorrhage.[1] In children, prophylactic platelet transfusion indicated when platelet count is less than 20,000/mm^3 with evidence of significant bleedings.[84]

Use of steroids

At present WHO is not recommending the use of steroids in the management of DHF/DSS.

There are two clinical trails in paediatric age group namely Sumarmo et al[82] which used hydrocortisone and Sampson Tassniyom et al[85] who used methyl prednisolone and found that the response in terms of mortality, duration of shock and amount of replacement fluids required same in both the study and control group.

Newer Drugs

The use of intravenous immunoglobin in DSS and efficacy of heparin in DIC have not yet been documented.

Prognosis[86]

Most of the dengue virus infections are asymptomatic while some present with nonspecific constitutional symptoms undifferentiated from other viral infections.

The mortality in DHF/DSS may be as high as 40-50 percent if left untreated. Early recognition of illness, careful monitoring and appropriate fluid

therapy alone has resulted in considerable reduction of mortality to 1-5 percent. Early recognition of shock is of paramount importance as the outcome of patient with DSS depends on the duration of shock.

With proper treatment recovery is fast and majority of the patients recover completely in 24-48 hours without any residual sequelae.

Prevention

Attenuated dengue viruses type 1,2,3 and 4 vaccines are under development. The possibility is that dengue vaccination may sensitize recipient so that ensuing dengue infection could result in hemorrhagic fever.

The basic preventive measures consist of control of Aedes aegypti mosquitoes, which breeds in and around human dwellings and flourish in water.

WHO global control programme recommends the followings:[87]

I. Selective integrated vector control with community and intersectoral participation.
II. Active surveillance based on a strong health information system,
III. Emergency preparedness.
IV. Capacity building and training.
V. Vector control.

Chapter 4

Methodology

METHODOLOGY

The present study was conducted in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum from January 2012 to December 2012.

Study design

The study design was cross sectional study.

Study period and duration

This study was conducted from January 2012 to December 2012 for a period of one year.

Place

The present study was conducted in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum a teaching hospital attached to Jawaharlal Nehru Medical College, KLE University, Belgaum.

Source of Data

The study was comprised of adult patients presenting with clinical features suggestive of dengue infection under Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum during the study period were included in the study.

Sample size

A total of 100 patients adult patients presenting with clinical features suggestive of dengue infection were selected for the study.

Sampling procedure

Considering the previous literature on the sensitivity of NS1 antigen detection kit for diagnosis of dengue virus infection the sample size was calculated using the following formula.

$n = 4\ z\alpha^2\ p\ q\ /\ d^2$

Where,

$z\alpha$ = Constant – 1.96

p = Prevalence (60%)

q = 100 – p = 40%

d = Absolute error considered as 20%

Hence,

$n = 4 \times 1.96^2 \times 60 \times 40 / 20^2$

n = 92.19

However to achieve uniform distribution, sample size of 100 patients was considered.

Selection criteria

Inclusion

- Age more than 12 years.

- History of documented fever of more than 38^0 C of less than seven days

plus

- Two or more signs and symptoms from the following;
    - Headache
    - Retro-orbital pain
    - Myalgia
    - Arthralgia
    - Rash
    - Hypotension
    - Bleeding manifestations

Exclusion

- Localised source of infection

- **Ethical clearance**

Period to the commencement, ethical clearance was obtained from Institutional Ethics Committee, Jawaharlal Nehru Medical College, Belgaum.

Informed Consent

The patients fulfilling selection criteria were explained about the nature of the study and a written informed consent was obtained (Annexure I).

Method of collection of data

Patients were interviewed for demographic data such as age sex and occupation were noted. Histories of similar complaints in past and current

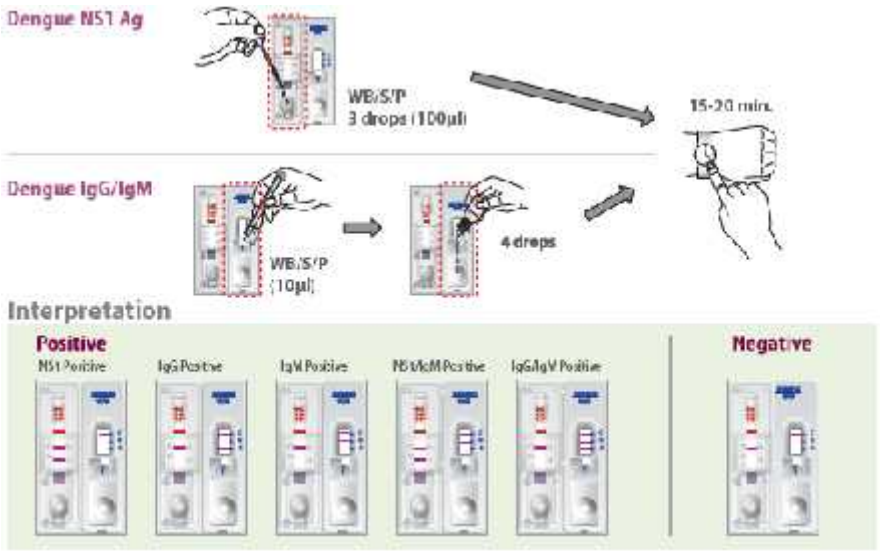
treatment were noted. Patients were subjected to a thorough physical examination, vitals (pulse rate, blood pressure and respiratory rate) and other clinical signs and symptoms of dengue fever. Systemic examination was carried out. These findings were recorded on a predesigned and pretested proforma (Annexure II).

Investigations

The selected patients underwent the following investigations.

- Complete blood count
- Renal function tests
- Liver function tests
- NS1 antigen testing for dengue
- Dengue IgM ELISA after 7 days
- Electrocardiography

<u>NS1 antigen testing for dengue</u>[88]



Under strict aseptic precautions, 3 mL blood was drawn by venipuncture. NS1 antigen testing for dengue was done using the Dengue NS1Ag+Ab Combo SD BIOLINE Dengue Duo kit manufactured by Standard Diagnostics Inc. It is a rapid, an in-vitro immunochromatographic, one step assay designed to detect dengue virus NS1 antigen.

The sensitivity and specificity of the kit was confirmed with IgM ELISA done later in the course of illness.

Outcome variables

The following parameters were assessed.

- Efficacy of the NS1 antigen test as compared IgM ELISA.
- Clinical features in early stage of dengue infection were noted.

Statistical analysis

The data obtained was coded and entered into Microsoft Excel Worksheet (Annexure III). The categorical data was expressed as rates, ratios and proportions and comparison was done using chi-square test. The continuous data was expressed as mean ± standard deviation (SD). The diagnostic accuracy of NS1 antigen testing, in predicting dengue infection was determined by sensitivity, specificity, positive predictive value and negative predictive value. Kappa agreement was used to correlate the agreements. A probability value (‘p’ value) of less than or equal to 0.05 was considered as statistically significant.

Chapter 5

Results

RESULTS

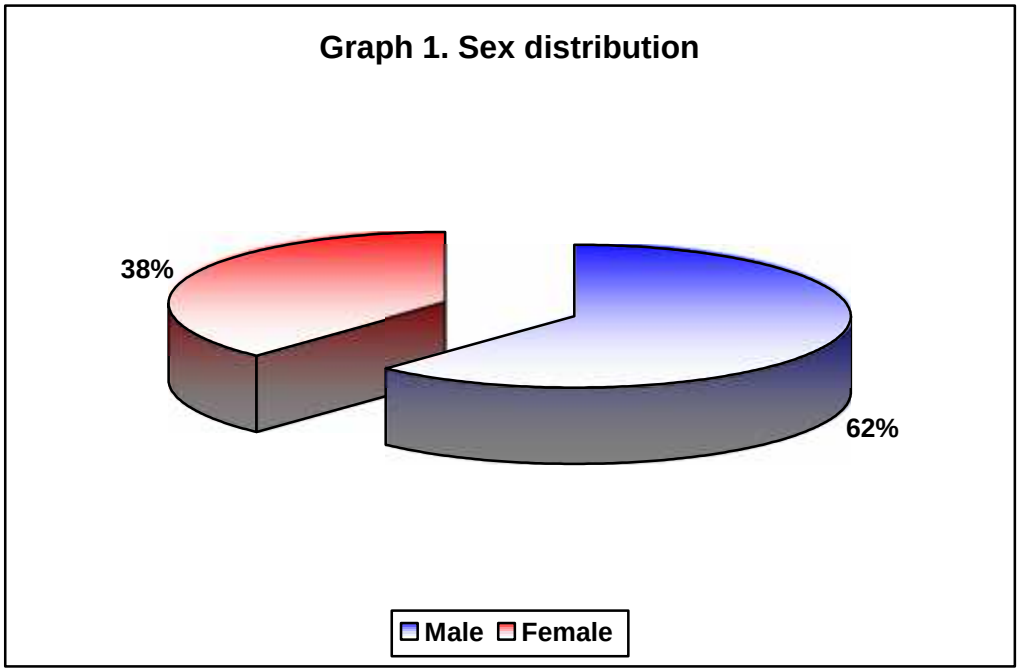
This one year cross sectional study was conducted in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum from January 2012 to December 2012.

A total of 100 adult patients presenting with clinical features of dengue infection were selected for the study.

Data obtained was coded and entered into the Microsoft Excel Spreadsheet. The data was analyzed and the final results and observations were tabulated as below:

Table 1. Sex distribution

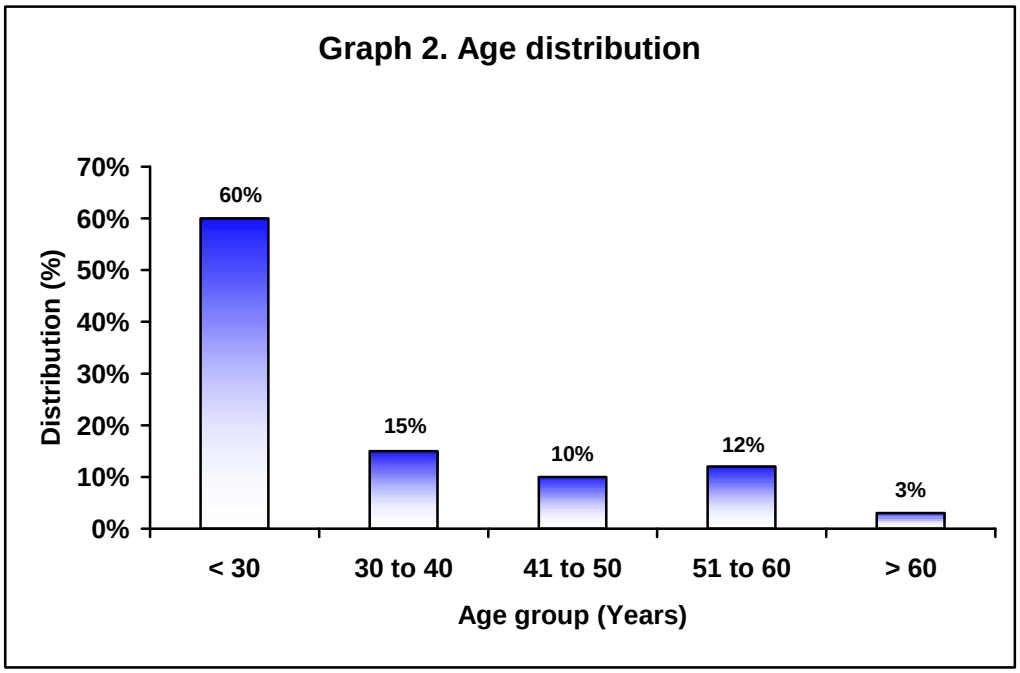
Sex	Distribution (n=100)	
	Number	**Percentage**
Male	62	62.00
Female	38	38.00
Total	**100**	**100.00**



In the present study 62% of patients were males and 38% of females. The male to female ratio was 1.63:1.

Table 2. Age distribution

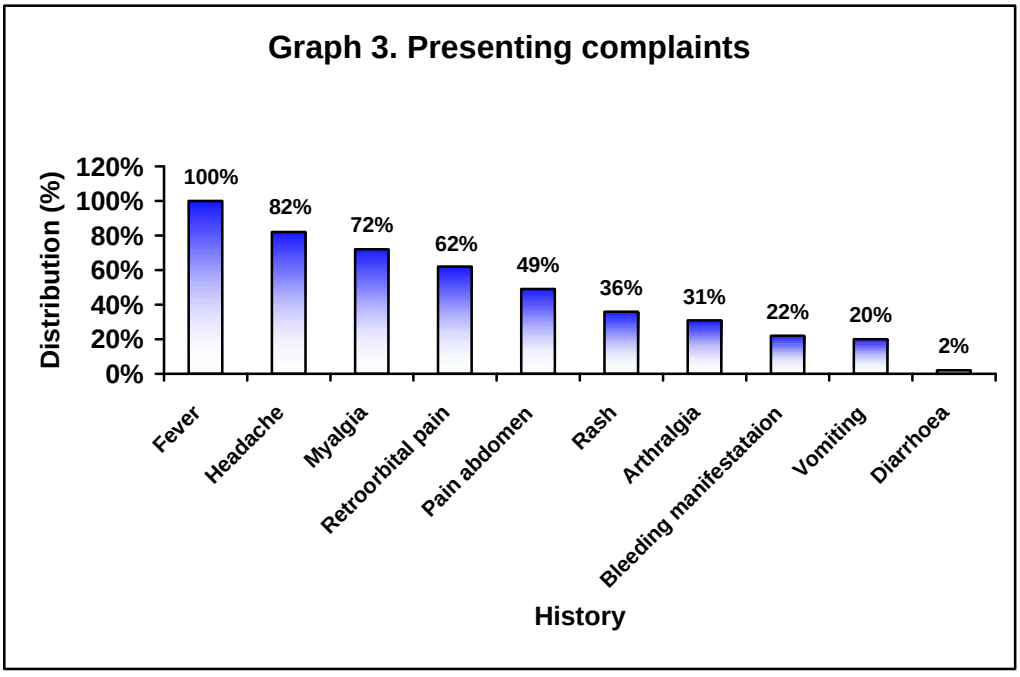
Age group (Years)	Distribution (n=100)	
	Number	**Percentage**
< 30	60	60.00
30 to 40	15	15.00
41 to 50	10	10.00
51 to 60	12	12.00
> 60	3	3.00
Total	**100**	**100.00**



In this study most of the patients (60%) were aged less than 30 years followed by 30 to 40 years (15%), 51 to 60 years (12%) and 41 to 50 years (10%). The mean age of the study population was 32.48 ± 13.87 years.

Table 3. Presenting complaints

History	Distribution (n=100)	
	Number	Percentage
Fever	100	100.00
Headache	82	82.00
Myalgia	72	72.00
Retroorbital pain	62	62.00
Pain abdomen	49	49.00
Rash	36	36.00
Arthralgia	31	31.00
Bleeding manifestation	22	22.00
Vomiting	20	20.00
Diarrhoea	2	2.00



In the present study all the patients presented with fever (100%). Headache, myalgia, retro-orbital pain and pain abdomen was reported by 82%, 72%, 62% and 49% of patients respectively. Bleeding manifestations and rashes were present in 22% and 36% of the patients respectively.

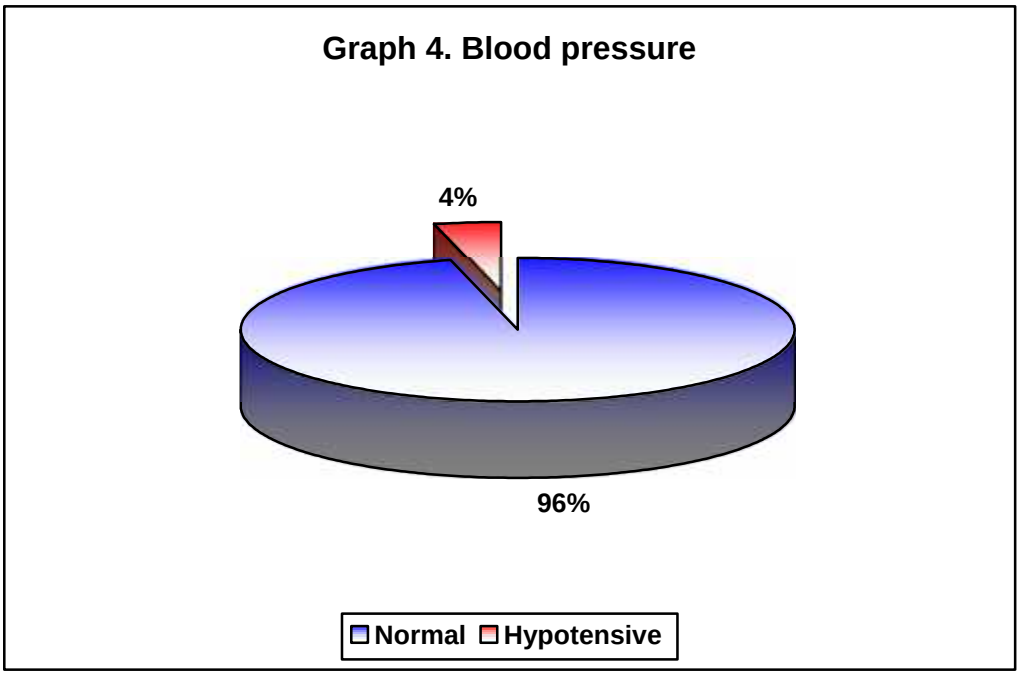
Table 4. Clinical features associated with dengue diagnosis using NS1 antigen test in patients evaluated upto 7 days of fever onset

Presenting complaints	NS1 positive		NS1 negative		p value
	Number	Percentage	Number	Percentage	
Headache	53	64.63	29	35.37	0.124
	15	83.33	3	16.67	
Myalgia	54	75.00	18	25.00	**0.016**
	14	50.00	14	50.00	
Retro orbital pain	47	75.81	15	24.19	**0.016**
	21	55.26	17	44.74	
Pain abdomen	40	81.63	9	18.37	0.069
	28	54.90	23	45.10	
Rash	31	86.11	5	13.89	**0.004**
	37	57.81	27	42.19	
Arthralgia	25	80.65	6	19.35	**0.004**
	43	62.32	26	37.68	
Bleeding	22	100.00	0	0.00	**<0.001**
manifestation	46	58.97	32	41.03	
Vomiting	17	85.00	3	15.00	0.068
	51	63.75	29	36.25	
Diarrhoea	2	100.00	0	0.00	0.327
	66	67.35	32	32.65	

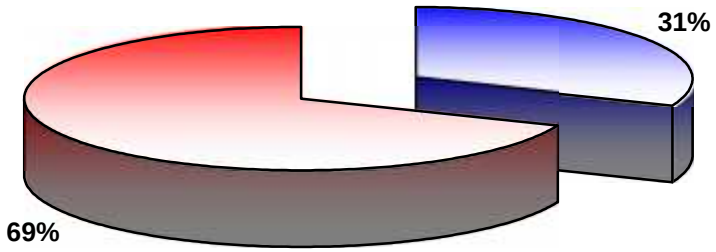
In this study we see that myalgia, retro orbital pain, arthralgia, rash and bleeding manifestations were significantly associated in patients who were NS1 positive.

Table 5. Clinical examination findings

Variables	Findings	Distribution (n=100)	
		Number	**Percent**
Blood pressure	Normal	96	96.00
	Hypotensive	4	4.00
	Total	**100**	**100.00**
Pulse rate	<60	31	31.00
	>60	69	69.00
Icterus	Present	16	16.00
	Absent	84	84.00
	Total	**100**	**100.00**
Oedema	Present	9	9.00
	Absent	91	91.00
	Total	**100**	**100.00**

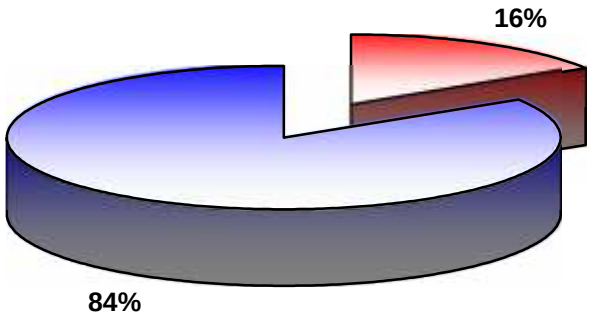


Graph 5. Pulse rate

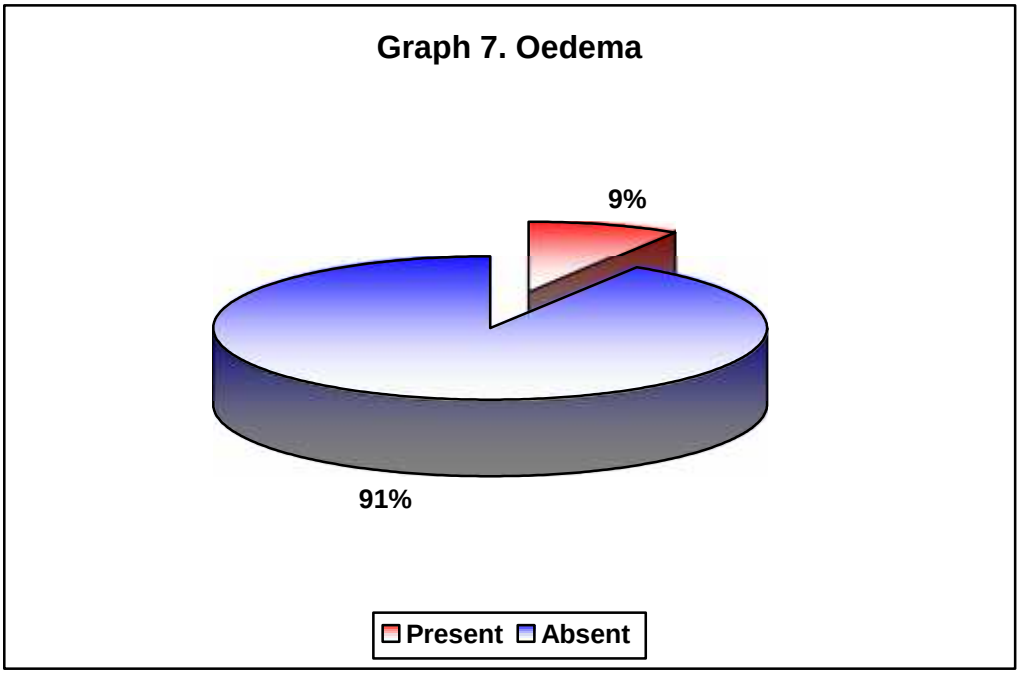


< 60 > 60

Graph 6. Icterus



Present Absent

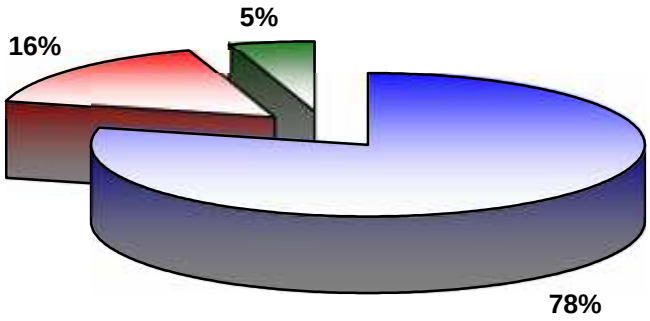


In the present study, the clinical examination revealed, hypotension in 4% of patients. Icterus and oedema was observed in 16% and 9% of patients respectively. Bradycardia was present in 31% of the patients.

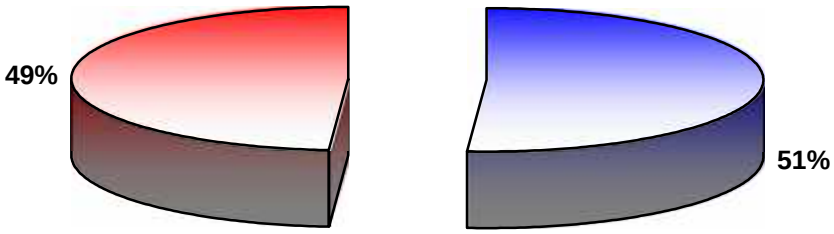
Table 6. Systemic examination findings

System	Findings	Distribution (n=100)	
		Number	**Percent**
Respiratory System	Normal	78	78.00
	Air entry ↓B/L bases	16	16.00
	B/L coarse crepitations	5	5.00
	Total	**100**	**100.00**
Per abdomen	Normal	51	51.00
	Tenderness	49	49.00
	Total	**100**	**100.00**
Organomegaly (hepatomegaly & spleenomegaly)	Present	20	20.00
	Absent	80	80.00
	Total	**100**	**100.00**
Central nervous system	Normal	95	95.00
	Altered sensorium	5	5.00
	Total	**100**	**100.00**

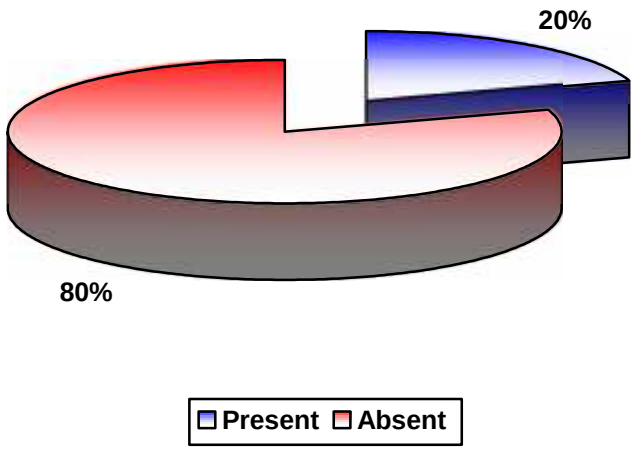
Graph 8. Respiratory system



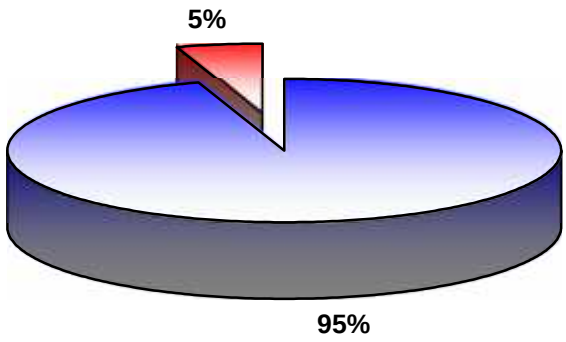
Graph 9. Per abdomen



Graph 10. Organomegaly (hepatomegaly and spleenomegaly)



Graph 11. Central nervous system

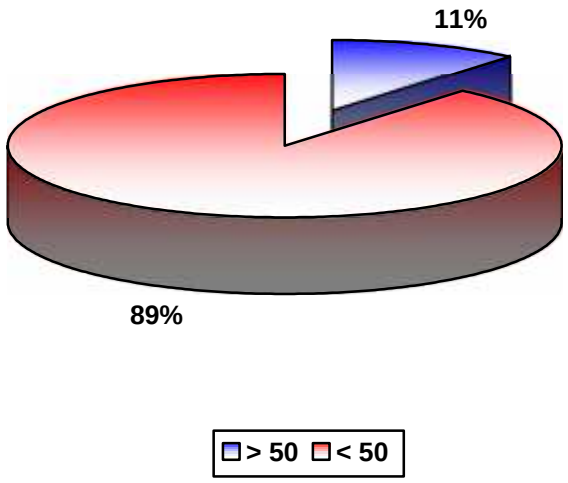


In this study, systemic examination revealed, decreased AE in bilateral bases and bilateral CC in 16% and 5% of patients in respiratory system. Abdominal tenderness was seen in 49% of patients and altered sensorium was present in 5% of patients.

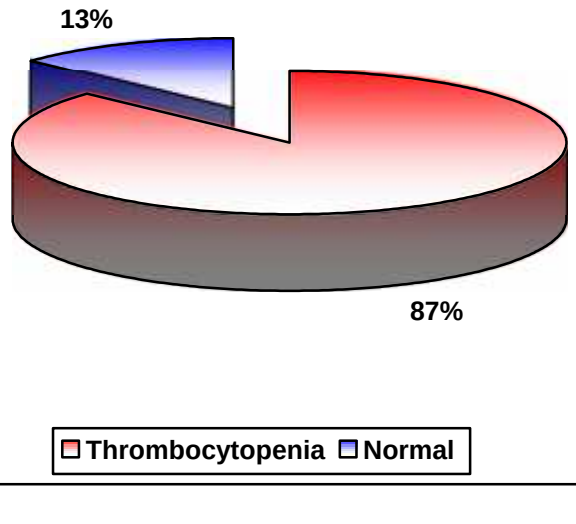
Table 7. Complete blood count

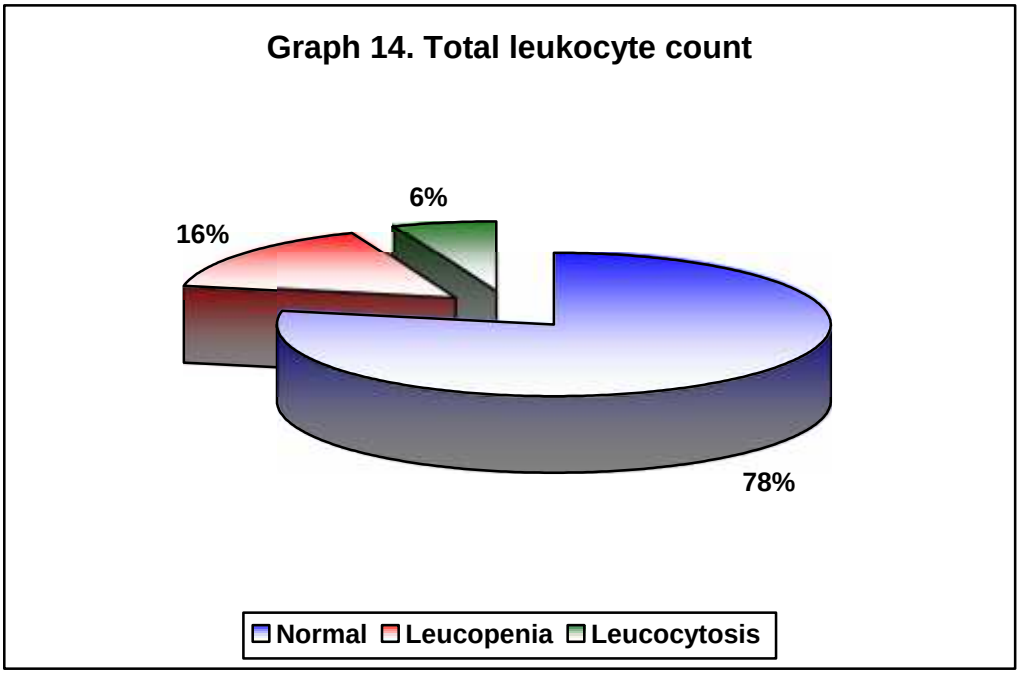
Variables	Findings	Distribution (n=100)	
		Number	**Percent**
Packed cell volume(%)	> 50	11	11.00
	< 50	89	89.00
	Total	**100**	**100.00**
	Mean ± SD	**42.40**	**8.30**
Platelet count (/mm^3)	Thrombocytopenia	87	87.00
	Normal	13	13.00
	Total	**100**	**100.00**
	Mean ± SD	**37120.57**	**32304.99**
Total leukocyte count (/mm^3)	Normal	78	78.00
	Leucopenia	16	16.00
	Leucocytosis	6	6.00
	Total	**100**	**100.00**
	Mean ± SD	**5500.00**	**2489.66**

Graph 12. Packed cell volume



Graph 13. Platelet count

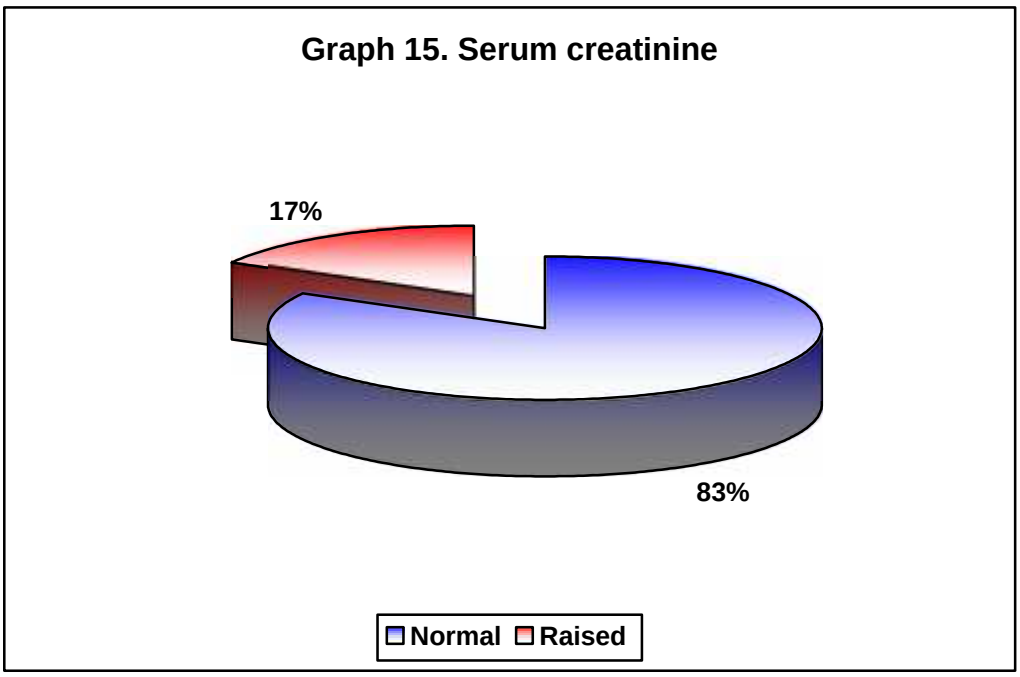


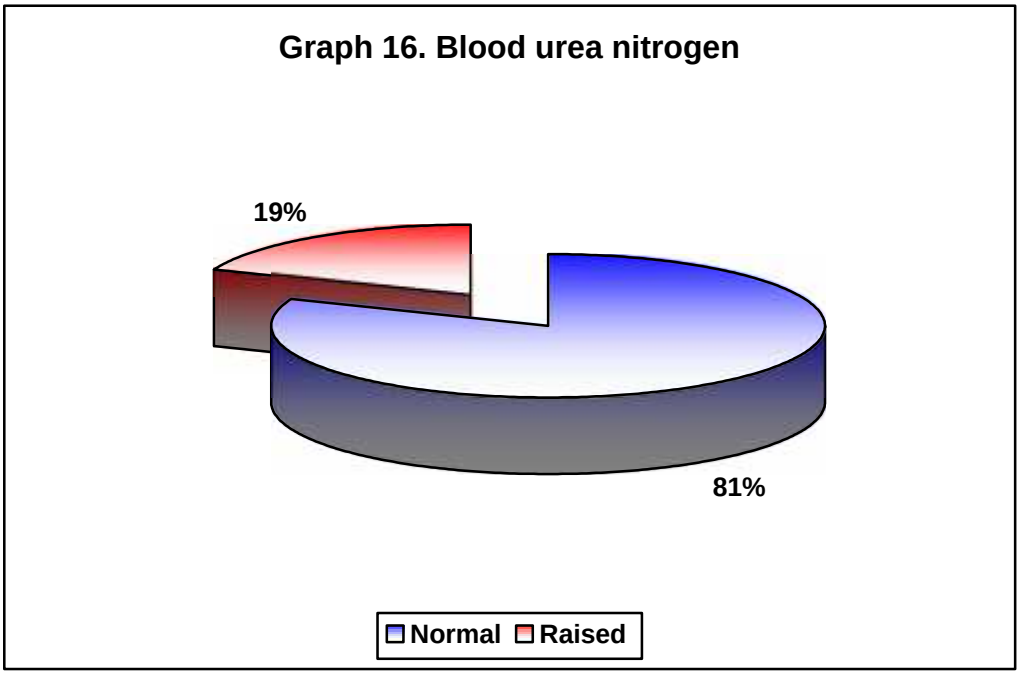


The haematocrit was increased in 11% of the patients while thrombocytopenia was present in 87% of the patients and leucopenia in 16% with mean values being 42.40 ± 8.30 cmm, 37120.57 ± 32304.99 /mm^3 and 5500 ± 2489.66 /mm^3 respectively.

Table 8. Renal profile

Variables	Findings	Distribution (n=100)	
		Number	**Percent**
Sr. Creatinine (mg/dL)	Normal	83	83.00
	Raised	17	17.00
	Total	**100**	**100.00**
	Mean ± SD	**1.19**	**1.07**
Blood urea nitrogen	Normal	81	81.00
(mg/dL)	Raised	19	19.00
	Total	**100**	**100.00**
	Mean ± SD	**33.84**	**35.82**



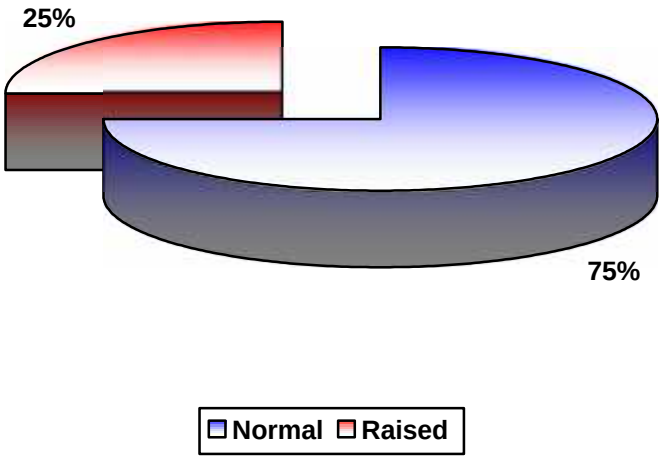


The present study showed raised serum creatinine in 17% of patients and raised blood urea nitrogen in 19% of patients. The mean serum creatinine and blood urea nitrogen were found to be 1.19 ± 1.07 mg/dL and 33.84 ± 35.82 respectively.

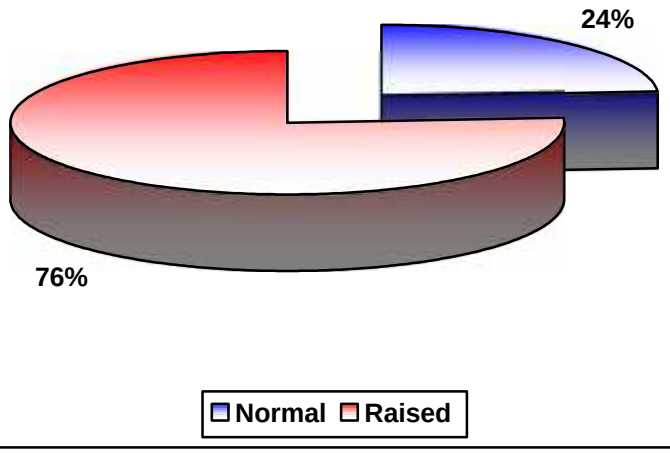
Table 9. Liver function test

Variables	Findings	Distribution (n=100)	
		Number	**Percent**
Total bilirubin (mg/dL)	Normal	75	75.00
	Raised	25	25.00
	Total	**100**	**100.00**
	Mean ± SD	**1.04**	**1.17**
SGOT (IU/L)	Normal	24	24.00
	Raised	76	76.00
	Total	**100**	**100.00**
	Mean ± SD	**122.04**	**172.27**
SGPT (IU/L)	Normal	49	49.00
	Raised	51	51.00
	Total	**100**	**100.00**
	Mean ± SD	**91.81**	**123.60**
Alkaline phosphatase	Normal	57	57.00
	Raised	43	43.00
	Total	**100**	**100.00**
	Mean ± SD	**144.39**	**91.99**

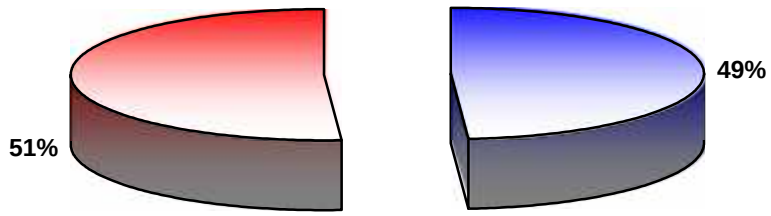
Graph 17. Total bilirubin



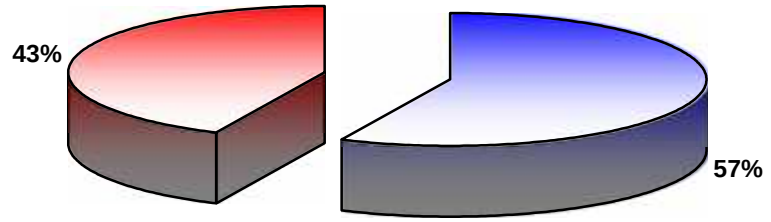
Graph 18. Serum glutamic oxaloacetic transaminase (SGOT)



Graph 19. Serum glutamic pyruvic transaminase (SGPT)



Graph 20. Alkaline phosphatase

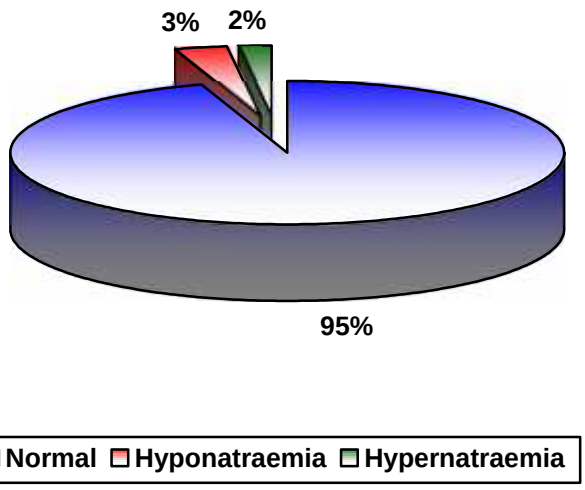


In this study, total bilirubin was raised in 25% of patients and the mean total bilirubin was found to be 1.04 ± 1.17 mg/dL. The SGOT and SGPT was abnormal in 76% and 51% of patients with mean values being 122.04 ± 172.27 and 91.81 ± 123.60 IU/L respectively. The alkaline phosphatase was raised in 43% of patients with mean values being 144.39 ± 91.99.

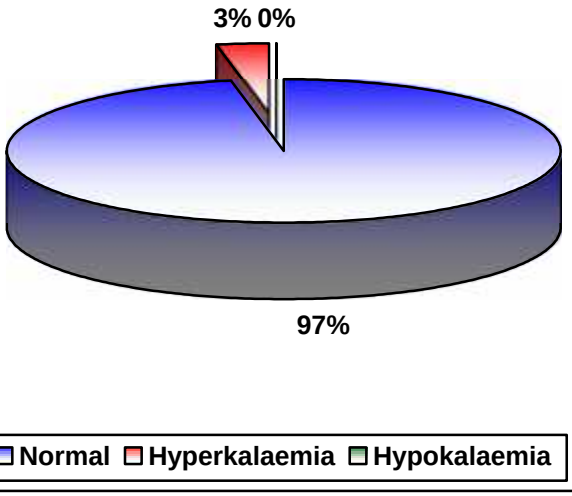
Table 10. Serum electrolytes

Variables	Findings	Distribution (n=100)	
		Number	**Percent**
Serum sodium (meq/L)	Normal	95	95.00
	Hyponatraemia	3	3.00
	Hypernatraemia	2	2.00
	Total	**100**	**100.00**
	Mean ± SD	**136.16**	**4.33**
Sr. potassium (meq/L)	Normal	97	97.00
	Hyperkalaemia	3	3.00
	Hypokalaemia	0	0.00
	Total	**100**	**100.00**
	Mean ± SD	**4.04**	**0.55**

Graph 21. Serum sodium



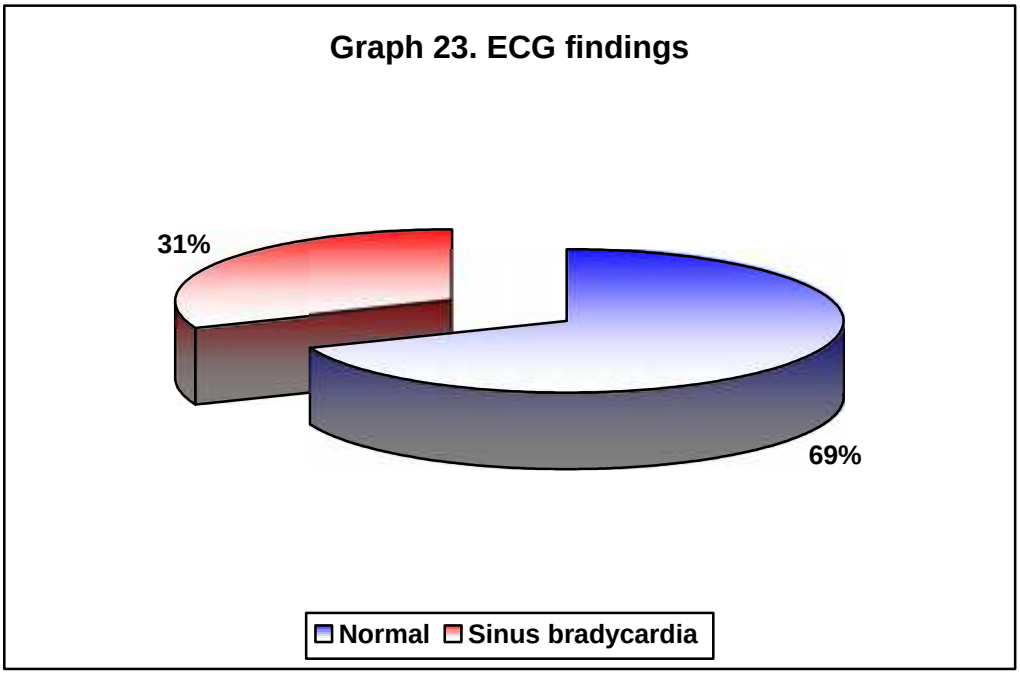
Graph 22. Serum potassium



In the present study serum sodium and serum potassium were found to be normal in 95% and 97% of the patients with mean values of 136.16 ± 4.33 and 4.04 ± 0.55 meq/L respectively. Hyponatraemia and hyperkalaemia were present in 3% patients each while hyperntraemia was noted in 2% of the patients

Table 11. ECG findings

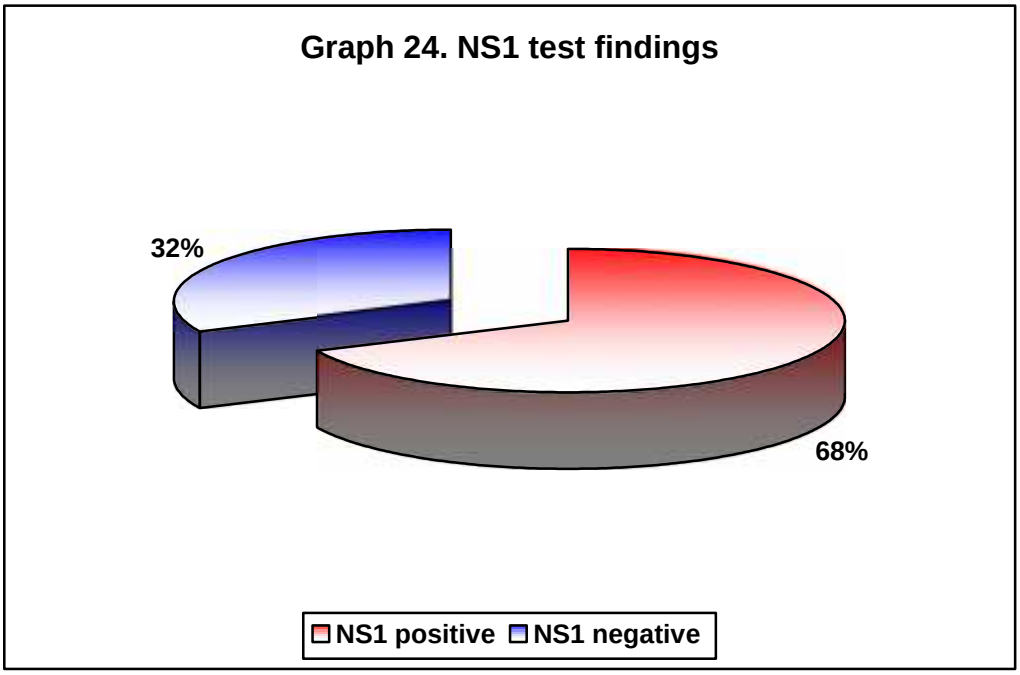
Findings	Distribution (n=100)	
	Number	**Percentage**
Normal	69	69.00
Sinus bradycardia	31	31.00
Total	**100**	**100.00**



In this study the ECG findings revealed sinus bradycardia in 31% of patients.

Table 12. NS1 test findings

Findings	Distribution (n=100)	
	Number	**Percentage**
NS1 positive	68	68.00
NS1 Negative	32	32.00
Total	**100**	**100.00**



In this study NS1 test was positive for dengue infection 68% of patients.

Table 13. Diagnostic accuracy of NS1 in comparison to IgM ELISA after seven days

NS1 test	IgM		Total
	Positive	**Negative**	
Positive	65	3	68
Negative	5	27	32
Total	70	30	100

p<0.001

Kappa= 0.813

SE of kappa = 0.063

95% confidence interval: From 0.689 to 0.937

The strength of agreement is considered to be 'very good'.

Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
92.86%	90%	95.59%	84.38%

In the present study 70 patients tested positive for IgM ELISA. Of these, 65 were positive for NS1 antigen test while five were negative. The sensitivity of NS1 in predicting dengue infection compared to IgM ELISA was 92.86% and specificity was 90% with 95.59% positive predictive value and 84.38% negative predictive value (p<0.001).

Table 14. Association of clinical signs with NS1 antigen test results

Variables		NS1 findings				p value
		Positive (n=68)		Negative (n=32)		
		No	%	No	%	
Pulse rate	< 60 /min	24	77.42	7	22.58	**0.175**
	> 60 /min	44	63.77	25	36.23	
Hypotension	Present	4	100.00	0	0.00	**0.161**
	Absent	64	66.67	32	33.33	
Icterus	Present	15	93.75	1	6.25	**0.016**
	Absent	53	63.10	31	36.90	
Oedema	Present	9	100.00	0	0.00	**0.031**
	Absent	59	64.84	32	35.16	
RS	Normal	51	62.96	30	37.04	**0.073**
	Air entry ↓ B/L bases	13	86.67	2	13.33	
	B/L Coarse crepitations	4	100.00	0	0.00	
PA	Normal	28	54.90	23	45.10	**0.004**
	Tenderness present	40	81.63	9	18.37	
CNS	Normal	63	66.32	32	33.68	**0.116**
	Altered sensorium	5	100.00	0	0.00	

Icterus and oedema were present significantly more in patients who tested positive for NS1 antigen ($p< 0.016$ and $p< 0.031$) respectively. Similarly, respiratory findings and abdominal tenderness were significantly more in NS1 positive patients ($p <0.05$).

Table 15. Association of CBC profile with NS1 antigen test results

Variables		NS1 findings				p value
		Positive (n=68)		Negative (n=32)		
		No	%	No	%	
PCV (%)	< 50	58	65.17	31	34.83	**0.084**
	> 50	10	90.91	1	9.09	
Platelet count	Normal	4	30.77	9	69.23	**0.002**
	Thrombocytopenia	64	73.56	23	26.44	
TLC	Leucopenia	13	81.25	3	18.75	**0.100**
	Normal	53	67.95	25	32.05	
	Leucocytosis	2	33.33	4	66.67	

In this study significantly higher number of patients with decreased platelet count (73.56%) had positive NS1 antigen test (p=0.002).

Table 16. Association of renal profile with NS1 antigen test results

Variables		NS1 findings				p value
		Positive (n=68)		Negative (n=32)		
		No	%	No	%	
Sr. Creatinine	Normal	52	62.65	31	37.35	**0.011**
	Raised	16	94.12	1	5.88	
BUN	Normal	52	64.20	29	35.80	**0.092**
	Raised	16	84.21	3	15.79	

In this study significantly higher number of patients (94.12%) had raised serum creatinine levels who were positive on NS1 antigen test for dengue infection (p=0.011)

Table 17. Association of liver profile with NS1 antigen test results

Variables		NS1 findings				p value
		Positive (n=68)		Negative (n=32)		
		No	%	No	%	
Total Bilirubin	Normal	48	64.00	27	36.00	**0.137**
	Raised	20	80.00	5	20.00	
SGOT	Normal	16	66.67	8	33.33	**0.872**
	Raised	52	68.42	24	31.58	
SGPT	Normal	29	70.73	12	29.27	**0.625**
	Raised	39	66.10	20	33.90	
Alkaline	Normal	40	70.18	17	29.82	**0.591**
phosphatase	Raised	28	65.12	15	34.88	

In the present study no statistically significant difference was observed with regard to total bilirubin, SGOT, SGPT and alkaline phosphatase in patients with positive and negative NS1 antigen test ($p>0.050$).

Table 18. Association of serum electrolytes with NS1 antigen test results

Variables		NS1 findings				p value
		Positive (n=68)		Negative (n=32)		
		No	%	No	%	
Serum sodium	Hyponatremia	3	100.00	0	0.00	**0.073**
	Normal	63	66.32	32	33.68	
	Hypernatremia	2	100.00	0	0.00	
Serum potassium	Hypokalemia	0	0.00	0	0.00	**0.228**
	Normal	65	67.01	32	32.99	
	Hyperkalemia	3	100.00	0	0.00	

In the present study no statistically significant difference was observed with serum electrolytes in patients with negative and positive NS1 antigen test (p>0.050).

Table 19. NS1 and ECG findings

Findings	NS1 Positive (n=68)		NS1 Negative (n=32)	
	No	%	No	%
Normal	44	63.77	25	78.12
Sinus bradycardia	24	35.23	7	22.58

p=0.176

In this study ECG revealed sinus bradycardia in 35.23% of patients with positive NS1 antigen test while 63.77% of patients had normal ECG who were positive on NS1 antigen test. This difference was statistically not significant (p=0.176).

Chapter 6

Discussion

DISCUSSION

Dengue is one of the most underreported tropical diseases and during epidemics, over reporting can occur at some hospitals. The lack of laboratory resources and the nonspecific clinical presentation of non-severe cases greatly contribute to this situation.

For any endemic disease, laboratory evidence is required for epidemiological surveillance. Virus isolation, molecular diagnosis using reverse transcriptase polymerase chain reaction (PCR) and serological methods have been used for laboratory confirmation of dengue infection. Viral isolation takes several days and is not available in many countries. Although PCR is a useful tool for identification, its widespread use for diagnosis of dengue infection is still limited due to high cost, especially in developing countries.

For primary dengue infection, capture IgM ELISA showed 80% of the patients had detectable levels by 5^{th} day, and 99% by the 10^{th} day.[89] Thus there is a lacunae in laboratory tests to diagnose dengue early within one week.

The need for simple point-of-care diagnostic tests has led to the proliferation of antibody-based Rapid Detection Tests (RDTs) for tropical infections such as dengue, leptospirosis, melioidosis, and malaria using the immunochromatographic test (ICT) format.

Immunochromatographic tests for the detection of dengue virus nonstructural protein 1 (NS1) antigen, IgM, IgG, and IgA antibodies have been developed and have found wide application because of their ease of use and

rapidity of results. These dengue RDTs are presented in the form of a lateral flow cassette that allows the flow of sample in a horizontal plane or a wick-style test that is performed in a tube and draws sample vertically by capillary action.

Dengue virus RDTs use a cocktail of dried antigens and colloidal gold-labelled monoclonal antibodies (specific for dengue NS1 antigen, IgM, IgG, or IgA antibodies) on a pad at the head of a nitrocellulose strip which is impregnated with either antidengue NS1 antigen, IgM, IgG, or IgA antibody lines. Test sample and running buffer are added to the pad which releases the colloidal gold from the pad and facilitates the mixing of the patient sample with the gold complex and facilitates the migration of the reagents and sample by capillary action along the nitrocellulose strip towards the anti-human IgM, IgG, or IgA antibody lines.

The presence of dengue virus NS1 antigen or IgM, IgG, or IgA antibodies is signified by the development of maroon lines in the location of the antibody lines. The dengue RDTs have the advantage that they can be performed in approximately 10–15 minutes and requires no specialized equipment or training, making them ideal for low-technology environments; however, this format has the weakness of subjective reading by the operator.

However, evaluation of the NS1 assay yielded moderately high sensitivity and very high specificity to dengue infection. NS1 being the newer test very few studies have reported the role of NS1 antigen detection in the early diagnosis of dengue virus infection. Hence the present study was undertaken to find out the

role of NS1 antigen test for early detection of dengue virus infection and to study the clinical features in early stage of dengue infection.

The present one year cross sectional study was conducted in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum. A total of 100 patients adult patients presenting with clinical features of dengue infection from January 2012 to December 2012 were studied.

In the present study slight male preponderance was seen that is, 62% of patients were males and the male to female ratio was 1.63:1. These findings were comparable with a study conducted by Agarwal et al[29] in which male to female ratio was 1.9:1. Another study conducted by Sharma et al[26] showed that male to female ratio was 3:1. The incidence of dengue is equal in males and females. However, fewer cases of dengue hemorrhagic fever and dengue shock syndrome have been reported in men than in women.

In this study most of the patients (60%) were aged less than 30 years followed by 30 to 40 years (15%), 51 to 60 years (12%) and 41 to 50 years (10%). The mean age of the study population was 32.48 ± 13.87 years. Dengue affects people of all ages. A retrospective study to review the changing epidemiology of the dengue between the years 2002 and 2008 by Chakravarthy A et al, reported presence of dengue in all the age groups of study population and noted predominance of adult population.[90]

In the present study all the patients presented with fever 100% followed by headache in 82%, myalgia in 72%, retro-orbital pain in 62% and pain

abdomen in 49% of patients. On clinical examination, icterus and edema was observed in 16% and 9% of patients that were statistically significant in NS1 positive patients (p 0.016 and p 0.031) respectively. Hypotension was seen in 4% of patients all of whom were NS1 positive. Abdominal tenderness was present in 49% of patients, arthralgia in 31%, rashes in 36% and neurological involvement was present in 5% of patients.

In our study bleeding manifestations were present in 22% of the patients of which majority presented with malena (8%). Bleeding manifestations were significantly more in NS1 positive patients ($p<0.001$).

Several studies[30,41-51] have reported different clinical features and complications of dengue fever. However, there is scarcity of data on the assessment of early clinical manifestations in dengue infection. A study from Singapore by Low JGH et al reported headache in 80%, myalgia in 69.2%, retro-orbital pain in 26% and pain abdomen in 11.6% of patients as early clinical symptoms in dengue infection.[89]

Recently, Kumar A et al.[50] in his record-based study conducted in a coastal district of Karnataka to study the clinical manifestations, trend and outcome of all confirmed dengue cases admitted in a tertiary care hospital assessed the laboratory confirmed cases from 2002 to 2008 from Medical Records Department (MRD). Of the 466 patients, the most common presentation was fever 462 (99.1%), followed by myalgia 301 (64.6%), vomiting 222 (47.6%), headache 222 (47.6%) and abdominal pain 175 (37.6%). The most common hemorrhagic manifestation was petechiae (67.2%). Of the 66 (14.1%) patients

who developed clinical complications, 22 (33.3%) had ARDS and 20 (30.3%) had pleural effusion.

In our study packed cell volume was increased in 11% of patients. Thrombocytopenia was seen in 87% of patients and leucopenia seen in 16% of the patients. Serum creatinine was raised in 17% of patients that was statistically significant (p 0.011) in NS1 positive patients and raised blood urea nitrogen was present in 19% of patients. The liver profile revealed, increased total bilirubin in 25% of patients and alkaline phosphatase was increased in 43% of patients.

The SGOT levels in dengue infection have a tendency to be greater than SGPT levels. This pattern is similar to that seen in alcoholic hepatitis but differs from the pattern in other viral hepatitis. The exact cause for this is unknown, but it is hypothesized that it may be due to excess release of SGOT from damaged liver cells during dengue infection. In our study we found elevation of liver enzymes with SGOT being higher than SGPT (mean SGOT 122.04 and mean SGPT 91.81). The SGOT and SGPT found to be increased in 76% and 51% of patients respectively. This abnormal pattern may be used as an early indicator of dengue infection.

In a study by Shivbalan S et al.[47] during 2004 on the predictors of spontaneous bleeding in dengue, a platelet count of less than 50,000 was found to be significantly associated with increased risk of bleeding. The other associated predictors of bleeding in the study conducted were prolonged PT, raised AST/ALT and haemoconcentration.

Serum sodium and serum potassium were found to be abnormal in 5% and 3% of patients respectively. The ECG findings revealed sinus bradycardia in 31% of patients.

In our study all patients in altered sensorium (5%) were positive for NS1 antigen. Our study showed 16% of the patients had air entry decreased bilateral bases and 5% had coarse crepitations bilaterally.

More recently Karoli R et al.[51] in their cross-sectional study at Lucknow during the monsoon and post-monsoon seasons in the year 2010 on 356 patients with suspected dengue fever found 138 (39%) had serologically confirmed dengue infection. Out of this Ninety-six (70%) patients had classical dengue fever while 42 (30%) had dengue hemorrhagic fever. The most common symptoms were headache (76%), abdominal pain (63%), vomiting (58%), rash (26%), and cutaneous hypersensitivity (16%). Hemorrhagic manifestations were present in 55 (40%) patients. Notably, 14% of patients had neurological involvement and 4% had acute hepatic failure. Study concluded that, dengue infection had varied and multi-systemic manifestations that can go unrecognized.

Laboratory tests are essential to provide an accurate diagnosis of acute dengue virus infection at patient presentation to a clinical setting so that appropriate treatment and patient management may be administered. In many dengue endemic settings, laboratory diagnostic resources are limited and simple rapid diagnostic tests (RDTs) provide opportunities for point-of-care diagnosis. The characteristics of the ideal diagnostic test are said to be defined by the ASSURED criteria: (1) Affordable by those at risk of infection; (2) Sensitive

(few false-negatives); (3) Specific (few false-positives); (4) User-friendly (simple to perform and requiring minimal training); (5) Rapid (to enable treatment at first visit) and Robust (does not require refrigerated storage); (6) Equipment-free; (7) Delivered to those who need it.[91]

In this study NS1 test was positive for dengue infection in 68% of patients and 70% of patients were positive for dengue infection on IgM. Of these, 65 were positive for dengue infection on NS1 while five were negative. The sensitivity of NS1 in predicting dengue infection compared to IgM was 92.86% and specificity was 90% with 95.59% positive predictive value and 84.38% negative predictive value ($p<0.001$). The strength of agreement was considered to be 'very good' based on Kappa statistics (Kappa 0.813; SE of kappa = 0.063; 95% confidence interval: From 0.689 to 0.937).

The NS1 antigen test for the diagnosis of acute dengue infection using admission samples had demonstrated considerable variation in sensitivity (49.8%–98.7%) but the specificities reported were more consistent with all being >90%[62]

Libraty et al. observed that a very high concentration of NS1 antigen within 72 hours of illness identified patients at risk of developing DHF [71]

A study by Datta S et al. in New Delhi to evaluate the efficacy of NS1 antigen (Ag) assay as an early marker for dengue virus (DV) infection concluded that, NS1 Ag assay holds promise in early diagnosis of dengue infection. When used in combination with MAC-ELISA on a single sample it significantly improves the diagnostic algorithm without the requirement of paired sera.[92]

A study by Singh MP et al, in Chandigarh compared IgM antibody detection with NS1 antigen for the diagnosis of acute dengue in 87 samples. NS1 antigen could be detected with good sensitivity (71-100%) till day 3 of fever, whereas IgM had a sensitivity of 0% to 50% at this time. On day 4 of illness, both the tests had comparative sensitivity. Beyond day 4, IgM antibody detection was superior to NS1. Both these diagnostic modalities were also compared with RT-PCR in 40 acute samples. NS1 detected additional 15 samples, which were missed by PCR. Study concluded that, NS1 antigen is an early diagnostic marker that is feasible in a routine diagnostic laboratory.[94]

In this study significantly higher number of patients with decreased platelet count (73.56%) had positive NS1 antigen test for dengue infection ($p=0.012$). A study by Kulkarni RD et al[94] from Karnataka, India tried to evaluate the association of platelet counts against NS1 and IgM/IgG in dengue infections. Serum samples from clinically suspected dengue cases were tested for NS1, IgM and IgG by immunochromatography-based test. Platelet counts were obtained for all positive cases and 150 dengue seronegative cases of fever that served as controls. Of 2104 samples tested, 320 were positive for one or more dengue parameters. Of the 320, 95 were positive for NS1 only, 161 showed IgM only while 9 showed IgG only. More than one marker was detected in the remaining 55 samples. Thrombocytopenia was more consistently associated whenever NS1 was detected compared to antibody detection ($p<0.001$). Study concluded that, inclusion of NS1 in the diagnosis of dengue increases the detection rate significantly. In cases of fever, thrombocytopenia is more consistently found in dengue positive rather than dengue negative subjects. It correlates well when NS1

and IgM are detected simultaneously. The findings of the present study were similar to the study done by Kulkarni RD et al.[94]

Overall the present study showed the usefulness of NS1 antigen test which is an excellent tool in addressing potentially fatal, epidemic prone dengue infection based on its easy and fast application compared to immunochromatography based dengue serology tests.

Similarly clinical features like retro orbital pain, myalgia, arthralgia, rash, bleeding manifestations along with laboratory findings like thrombocytopenia, SGOT>SGPT strongly support the diagnosis of dengue fever.

The limitations of this study were, smaller sample size, and NS1 antigen test was not evaluated considering PCR, which has higher sensitivity than NS1 antigen tests though which was beyond the scope of this study. Further studies comprising of large sample size with Polymerase Chain Reaction (PCR) would further explore the role of NS1 antigen testing in the diagnosis of dengue infection.

Chapter 7

Conclusion

CONCLUSION

Identification of dengue infection early during an acute phase of illness is valuable for the clinician due to increased risk of progression to life threatening complication and therefore of utmost importance to reduce the case fatality rate.

Early identification of dengue infection in acute phase sera using NS1 antigen rapid detection test is valuable in terms of disease progression and mortality.

In our study NS1 RDT showed promising results with sensitivity and specificity of 92.86% and 90% respectively when compared with IgM ELISA done later in the course of illness. However in highly suspected cases of dengue infection clinical management should not rely on negative serological results.

Considering only clinical features, in our study we found that presence of retro orbital pain, myalgia, bleeding manifestations, rashes and arthralgia were significantly (p value <0.05) more in patients who were NS1 positive.

Patients with dengue fever are more prone to have liver enzyme derangement as found in our study and preferentially high SGOT may serve as an early indicator of dengue infection.

Similarly thrombocytopenia was more significant (p value 0.002) in NS1 positive patients and it is a very important indicator of prognosis in dengue fever.

Thus we conclude that dengue infection, which possesses serious public health problem, can be diagnosed early with the help of clinical features like

retro-orbital pain, myalgia, bleeding manifestations, thrombocytopenia, SGOT greater than SGPT that is supported by detection of NS1 antigen.

Chapter 8

Summary

SUMMARY

Dengue virus infection has emerged as a major public health concern across the globe in terms of case fatality rate and public health cost. If identified early and managed accordingly there can be significant reduction in mortality and morbidity associated with it. The present study was aimed to find out the role of NS1 antigen for early detection of dengue virus infection versus ELISA and to study the clinical features in early stage of dengue infection.

The present one-year cross sectional study was conducted in the Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum. A total of 100 adult patients presenting with clinical features of dengue infection from January 2012 to December 2012 were studied. The diagnosis was confirmed with NS1 RDT whose efficacy was later tested with IgM ELISA.

In this study young males were predominantly affected. NS1 positivity was 68%. The sensitivity of NS1 in predicting dengue infection compared to IgM was 92.86% and specificity was 90% with strength of agreement considered to be 'very good' based on Kappa statistics. Clinical features like retro orbital pain, arthralgia, myalgia, rashes and bleeding manifestations were significantly more in patients who were NS1 positive. Similarly icterus, edema, hypotension and altered sensorium were significantly associated with NS1 positivity. Interestingly, liver profile showed SGOT more than SGPT. Thrombocytopenia and raised creatinine were significantly more in NS1 positive patients.

Early identification of dengue infection could help clinicians institute acceptable case management and to identify patients who should be closely monitored for signs of plasma leakage. This study will help clinicians diagnose dengue early with the help of clinical features and simple diagnostic test, which will help reduce significant burden of the disease.

Chapter 9

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Annexures

Annexure I

ANNEXURE I – CONSENT FORM

Title Of Research Study: ROLE OF NS1 ANTIGEN DETECTION IN EARLY DIAGNOSIS OF DENGUE VIRUS INFECTION

Principal Investigator:-
Dr. *** ***** *****
Post Graduate Student,
Department Of General Medicine,
J.N.Medical College, Belgaum.

Introduction and Purpose:-

Diagnosing dengue early is challenging because the initial symptoms of dengue infection are often non-specific and serological tests, which are the mainstay of current laboratory diagnosis, confirm dengue late in the course of illness. The complications of Dengue fever usually occur between 3-7 days of fever.So an early diagnostic test is necessary to detect and prevent complications.

Procedure:

If you agree to be part of the research study, you will be asked the relevant history and will be subjected to relevant clinical examination and investigations. You will also have to give blood and urine samples for the necessary investigation.

Risk and Benefits:

The only risk and possible discomfort you might get is while taking blood from my arm for the investigations. It may cause swelling, pain, redness, bruising or infection (rarely happens) at the site from where the blood is drawn.

Alternatives:

Taking part in this study is voluntary. You may choose not to take part in this study, or if I decide to take part I can later change my mind and withdraw from the study. Your decision will not change the present or future health care or other services that you receive. The study doctor or sponsor may stop your participation in this study at any time. If you choose not to take part in the study, you will receive the standard treatment for patients with your condition.

Privacy and Confidentiality:

All information collected about you during the course of this study will be kept confidential to the extent permitted by law. The code numbers will identify you in this research record. Information from this study may be published but your identity will be confidential in any publication.

Institution / Sponsor's policy:

Does not apply to this research

Financial incentives for participation:

You will not be paid / offered any gifts /incentives for participating in the study.

Authorization to publish the results:

The results of the study would be forwarded to the KLE University, Belgaum as part of requirement towards the completion of MD degree, review and publishing.

In case of the queries during study or in future you may contact following persons,

1. Dr. *** *****, Chairman,
J.N.M.C Ethical Committee for Human Research,
Phone number: **** ******.
Extn: ****

2. Dr. ****** ******,
Professor& HOD,
Department of Medicine,
JNMC, Belgaum.
Phone No: ***** ******,
Extn: ****/****

3. Dr. ***** ******,
Assoc Professor & Head of Unit,
Dept of General Medicine,
JNMC, Belgaum.
Phone No.: ***** ******

4. Dr. *** ***** *****,
Investigator,
PG in General Medicine,
JNMC, Belgaum.
Phone No.: ***** ******

Consent Statement

I voluntarily agree to take part in this study by signing below. I may withdraw at any time. I am not giving up any of my legal rights by signing this form. My signature below indicates that I have read, or it has been read to me, this entire consent form, and have had all my questions answered.

Name of the Participant : _____ Signature / Thumb print _____

Name of the Witness _____ Signature/ Thumb print _____

Investigator Name: _____ Signature :_____

Date: Place:

Annexures

Annexure II

ANNEXURE II – PROFORMA

Case No:

NAME: **AGE/SEX:**

IP No. **ADDRESS:**

OCCUPATION:

COMPLAINTS AT PRESENTATION: **YES** **NO**

1. History of documented fever of >38°C of less than 7 day
2. Headache,
3. Retro-orbital pain,
4. Myalgia,
5. Arthralgia,
6. Rash,
7. Hypotension,
8. Bleeding manifestations
9. Any localized source of infection

PAST HISTORY:

History of similar complaints in the past

TREATMENT HISTORY:

Received any treatment for similar complaints in the past

PHYSICAL EXAMINATION:

GENERAL CONDITION:

Pallor: Yes/No

Icterus: Yes/No

Lymphadenopathy: Yes/No

Cyanosis: Yes/No

Clubbing: Yes/No

Edema: Yes/No

VITALS:

Temperature:

Pulse:

Respiratory rate:

Blood pressure:

SYSTEMIC EXAMINATION:

R. S.:

C.V.S.:

P.A.:

C.N.S.:

Annexures

Annexure III

ANNEXURE III – MASTER CHART

-	-Absent
+	-Present
AE↓	-Air entry decreased bilateral bases
Alt Sens	-Altered Sensoriun
BUN	-Blood urea nitrogen
CC	-Coarse crepitations
DBP	-Diastolic Blood Pressure
dL	- Deci litre
E	-Epistaxis
GB	-Gum Bleeding
gm	- Gram
H	-Haematuria
HPT	-Hepatomegaly
IgM	-Immunoglobin M ELISA
IU	-International Unit
L	- Liter
meq	- Milli equivalent
mg	- Milli gram
ML	-Malena
N	-Normal
NS1	-Non Structural Antigen Test
PVB	-PV bleeding
SB	-Sinus bradycardia

SBP	-Systolic Blood Pressure
SGOT	- Serum glutamic oxaloacetic transaminase
SGPT	- Serum glutamic pyruvic transaminase
SPL	-Spleenomegaly
Sr.	- Serum
TEND	-Tenderness
TLC	-Total leucocyte count