A STUDY ON INCIDENCE AND SEROTYPING OF DENGUE IN A TERTIARY CARE HOSPITAL

Dissertation Submitted to
The Tamil Nadu Dr. M.G.R. Medical University

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For the award of the degree of

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BRANCH – IV

MADRAS MEDICAL COLLEGE
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MARCH 2009

CERTIFICATE

This is to certify that this dissertation titled **"A STUDY ON INCIDENCE AND SEROTYPING OF DENGUE IN A TERTIARY CARE HOSPITAL"** is a bonafide record of work done by **Dr. C.S. SRIPRIYA,** during the period of her Post graduate study from June 2006 to March 2009 under guidance and supervision in the Institute of Microbiology, Madras Medical College and Government General Hospital, Chennai-600003 in partial fulfillment of the requirement for **M.D. Microbiology** Degree Examination of The Tamilnadu Dr. M.G.R. Medical University to be held in March 2009.

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DECLARATION

I declare that the dissertation entitled ***"A STUDY ON INCIDENCE AND SEROTYPING OF DENGUE IN A TERTIARY CARE HOSPITAL"*** *submitted by me for the degree of M.D. is the record work carried out by me during the period of* ***October 2006 to October 2007*** *under the guidance of* ***Dr.THASNEEM BANU.S, M.D.,*** *Additional Professor of Microbiology, Institute of Microbiology, Madras Medical College, Chennai. This dissertation is submitted to The Tamilnadu Dr.M.G.R. Medical University, Chennai, in partial fulfillment of the University regulations for the award of degree of M.D., Branch IV (Microbiology) examination to be held in March 2009.*

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CONTENTS

Introduction

INTRODUCTION

We stand on the brink of an era in which millions of people are likely to be safer from some of the most terrifying and maiming diseases. But, some new and previously unknown diseases continue to emerge, which are often labelled as 're-emerging diseases'. These, amount to a crisis that is a challenge for the public health system in many parts of the world [86].

Viral haemorrhagic fevers are becoming increasingly common in the tropics and subtropics. Dengue fever is currently the most important arthropod borne viral disease because of its widespread distribution in more than 100 countries and its potential for extensive outbreaks of life-threatening disease. Two-fifths of world's population or 2500 million people are now at risk for dengue and every year approximately 50 million new cases occur worldwide.[63]

Dengue virus was first isolated in India in the year 1945 and is endemic in both urban and semi-urban areas. Dengue fever has struck again in India and cases of dengue fever (DF)/dengue haemorrhagic fever (DHF) have been reported from various parts of the country during the last 4 decades. [86]

During the epidemics of dengue, attack rates among susceptibles are 40-90% and an estimated 5,00,000 cases of DHF require hospitalization each year, of whom a very large proportion are children. [7]

Dengue virus, belonging to the genus *Flavivirus* and Family *Flaviviridae,* are mosquito borne viruses and the principal vector, *Aedes aegypti* is a day-biting mosquito of public importance that breeds in natural or artificial waters.

Dengue illnesses are caused by any one of the four serologically related viruses designated as DENV-1, DENV-2,DENV-3 and DENV-4.[91] Infection with any one of these serotypes mostly causes a mild, self-limiting febrile illness (Classical dengue fever), however, a few cases develop severe life threatening dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). [91]

Classical dengue fever is seen 4-6 days after an infective mosquito bite, with sudden onset of fever (often biphasic), severe headache, chills, generalized pains in muscles and joints, often associated with maculopapular rash. There is leucopenia, relative lymphocytosis, thrombocytopenia and haemorrhagic manifestations may occur. [96]

DHF and DSS are severe forms of the disease characterized by sudden onset of fever and nonspecific signs and symptoms. The critical stage of DHF occurs 24 hrs before to 24 hrs after the temperature falls to or below normal. During this time, haemorrhagic manifestations usually occur and signs of circulatory failure may appear. Laboratory tests show thrombocytopenia and evidence of vascular leak syndrome. Hypovolemia, shock and death may occur in case of DSS. [96]

Primary infection with one of the four serotypes confers lasting immunity to that serotype. Secondary infection with a different serotype is associated with an increased risk of DHF. [9]

The diagnosis of DF and DHF is made on clinical and epidemiological grounds. In some areas, DHF overlaps with the distribution of other viral haemorrhagic fevers, thereby causing a confusion in the diagnosis.

Therefore, serological diagnosis by detection of IgM and IgG antibodies to dengue in

the serum is essential for monitoring the treatment. Commercial kits are available, which can help in differentiating between primary and secondary dengue infections. A rapid dengue detection test kit is used for the preliminary diagnosis. ELISA tests are very useful in dengue serology. They detect IgM and IgG in the serum and thus are able to distinguish primary and secondary infection.

Since the occurrence of dengue infections and complications like DHF and DSS are increasing, this study was conducted to study the incidence of dengue infections, to evaluate the seropositivity and to determine the serotype of dengue virus in a tertiary care setup, thereby to create awareness about the preventive measures to be taken by the general public and the health care system, and to improve our infrastructure for diagnosing and treating dengue infections.

Review of Literature

REVIEW OF LITERATURE

HISTORY

Dr. Benjamin Rush's description of a Philadelphia epidemic in 1780 was the earliest description of dengue, the break-bone fever. Subsequently, sporadic outbreaks were reported throughout the tropics and subtropics.[93]

Although dengue fever had been described in the 18th century, the virus was isolated only during World War II.[93]

Clinical description of dengue complicated by hemorrhages, shock and death were reported in outbreaks in Australia in 1897, Greece in 1928 and in Formosa in 1931. Mosquito borne transmission of infection by *Aedes aegypti* was demonstrated in 1903 and its viral etiology in 1906. Sabin isolated the virus in 1944 and established the existence of dengue viral serotypes.[75]

Between 1944 and 1956 it was shown that four distinct viruses, designated dengue virus types 1-4 were responsible for the same clinical syndrome. In 1956, a severe form of the disease, dengue hemorrhagic fever/dengue shock syndrome were described for the first time.[93]

After World War II, the start of a pandemic with intensified transmission of multiple viral serotypes began in Southeast Asia, leading to outbreaks of dengue hemorrhagic fever.[75]

In the last 25 years, a similar pattern of intensified viral transmission and increased

dengue hemorrhagic fever incidents has been established in south west Asia, the Americas and Oceanic, fueled by secular changes toward urbanization, population growth and mobility.[75]

The Indian encounter with dengue and dengue hemorrhagic fever is interesting and intriguing. The first major epidemic illness compatible clinically with dengue occurred in Madras in 1780 with later spread to all over the country. The dengue virus was first isolated in Japan in 1944, but the one isolated in Calcutta in 1944 from the blood of US soldiers was considered as a first report for a longtime[7.] The epidemics from India include those from Calcutta(1963), Vishakapattanam(1964), West Bengal(1968), Ajmir(1969), Kanpur (1969), Delhi (1970), Rajasthan (1985) and Delhi in 1996. [102,44]

Dengue/ DHF is widely prevalent in India, and all the 4 serotypes are found in the country. It is reported from 15 states/ Union Territories since 1996. In Southern India, the disease has been reported in TamilNadu, Karnataka, Andhra Pradesh and Kerala. [67]

ETIOLOGY

Dengue viruses are arboviruses belonging to the Genus *Flavivirus* and Family *Flaviviridae.*

CLASSIFICATION

Dengue fever is caused by four antigenically related but distinct viruses (serotypes 1 to 4) distinguished by neutralization tests.[88] Infection with Serotype 1 followed by Serotype 2 is more dangerous than Serotype 4 followed by Serotype 2.[25] At the genomic level, strains of dengue viruses belonging to same serotype are >90% homologous whereas homology across serotypes is approximately 65%. Nucleotide sequencing of the ‘E gene’ has provided a

means of classifying unique genotypes of each dengue virus serotype.[93]

Distinct genotypes have evolved in different geographic regions, and genotyping thus provides a means of determining the origin and spread of epidemics.[93]

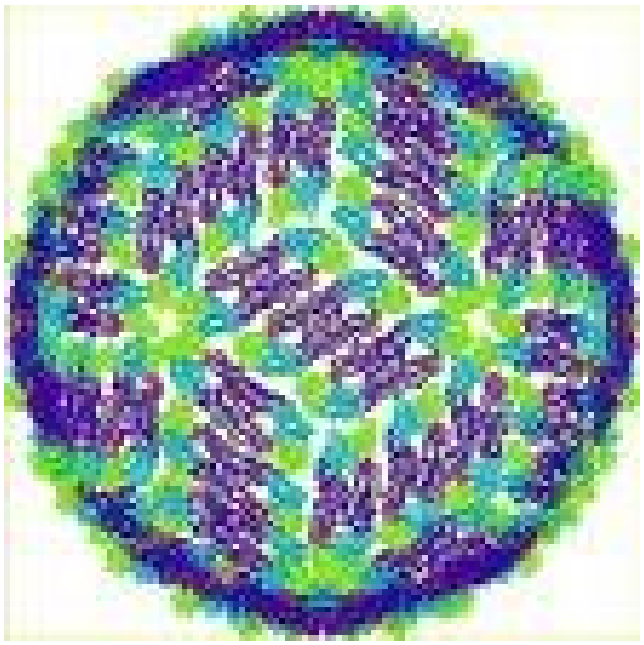
Genotypic classification of dengue viruses[93]

Serotype	Genotype	Distribution
1	I	• Thailand, Indonesia, Malaysia,Pacific Islands
	II	• Thailand, arribean, Africa,Pacific Islands
	III	• Thailand,Philippines(Includes Prototype Hawaii)
2	I	• Thailand,Burma,Malaysia,Vietnam,Caribbean (includes prototype New Guinea C)
	II	• Srilanka, Seychelles
	III	• Africa
	IV	• Africa
	V	• Americas
	VI	• Pacific Islands
3	I	• Indonesia, Malaysia,Pacific Islands
	II	• Thailand, Malaysia, Indonesia, Burma, Vietnam, Philipines(includes prototype H87)
	III	• Carribean, Pacific Islands
	IV	• Thailand
4	I	• Philippines,Southeast Asia,Africa, America, Pacific Islands (includes prototype H241).

MORPHOLOGY

Dengue virus particles are 40 to 50 nm in diameter and have a spherical nucleocapsid surrounded by a lipid bilayer envelope with small surface projections representing E-glycoprotein dimers anchored to virus membrane. The lipid envelope is covered densely with

surface projections comprising 180 copies of the membrane and 180 copies of the envelope glycoproteins.[39]



Colour Plate:1; Structure of Dengue virus.

GENOMIC STRUCTURE:

The genome is a single stranded RNA containing approximately 11,000 nucleotides, composed of short 5' noncoding region, a single long open frame containing more than 10,000 nucleotides and the 3'noncoding terminus.

The long open reading frame encodes three structural proteins at the 5'end which are the capsid (C), premembrane (preM) and envelope (E) proteins. These are followed down stream by 7non-structural (NS) proteins in the sequence NS1, NS2a-NS2b-NS3-NS4a-NS4b-NS5. [93]

The structural proteins are included in the mature virion, whereas the NS proteins play various roles in virus replication and polypeptide processing.

The E proteins are organized as dimers, paired horizontally head to tail, on the virion surface. The E protein exhibits important biologic properties including viral cellular attachment, endosomal membrane fusion and the display of sites mediating hemagglutination and viral neutralization. [27]

OTHER NAMES

Break-bone fever [93],Saddle back (biphasic) fever[93]

RISK FACTORS FOR DENGUE HEMORRHAGIC FEVER

The risk factors for dengue hemorrhagic fever are:[7]

- Infestation with *Aedes* mosquito
- Hot and humid climates enhancing mosquito breeding.
- Mosquito density
- Presence of all four serotypes of dengue virus with secondary infection in the host.
- Water storage pattern in the houses & Population density
- Larger movement of people towards urban areas.

EPIDEMIOLOGY

Dengue virus occurs worldwide in tropical region, their distribution determined by the presence of the principal mosquito vector, *Aedes aegypti*. In tropical areas the vector is alive year-round and dengue occurs throughout the year with increased transmission during rainy season. This is due to higher mean temperatures and the attendant shorter extrinsic incubation period (the interval between feeding on an infectious blood and the ability to transmit on refeeding) in the vector and to higher humidity and enhanced survival of adult mosquitoes[93]

In temperate zones the transmission is limited to summer months. The distribution and abundance of the vector are now more restricted due to improved sanitation and use of piped water, but the potential exists for introduction and spread of the virus in temperate areas.[93]

It is estimated that over 2.5 billion people inhabiting the tropical areas are at risk of dengue infection.[14]

Dengue infections are most prevalent in Southeast Asia where all four serotypes are continuously present. In recent years, the Indian sub continent, southern China and Taiwan have experienced epidemics. [56]

In areas of Southeast Asia with hyper endemic infection, the annual incidence of infection is 10 to 20%, and most children have experienced at least one dengue infection by age of 7 years.[18]

The immunity acquired after infection with one serotype confers full (probably lifelong)

protection against re-infection with that serotype, but predisposes to more severe disease (DHF) on sequential infection with another dengue infection. [93]

The intensification of dengue transmission in tropical cities where growing population live under crowded conditions can be understood in view of the close relationship of *Aedes aegypti* to humans.[77]

After the female mosquito feeds on a viraemic person, viral replication in the mosquito over one to two weeks (extrinsic incubation period) occurs before it can transmit the virus on subsequent feeding attempts. Feeding attempts may occur several times a day over the insects' lifetime of one to four weeks. Adult mosquito shelter indoors and bite during one to two hour intervals in the morning and later afternoon. In areas with endemic transmission, one of every twenty hours may contain an infected mosquito. [41]

The dissemination of dengue virus by viremic travelers have been facilitated by increased mobility of the people living within endemic areas and internationally by burgeoning air travel. [29]

The 1996 epidemic in India was mainly due to dengue type 2 virus while the 2003 epidemic appears to be mainly type3 virus.[7]

INACTIVATION BY PHYSICAL AND CHEMICAL AGENTS:

Dengue viruses are rapidly inactivated by ionic and nonionic detergents, trypsin, UV light, gamma–irradiation, formaldehyde, beta-propiolactone and most disinfectants including chlorine, iodine, phenol and alcohol. [93]

The viruses are optimally stable at temperatures below -70˚C and are rapidly inactivated in blood and other liquids within 30 minutes at 50˚C. Dengue viruses are most stable at pH 8.4 -8.8 and are rapidly degraded at lower pH. Sensitivity to acid, bile, lipases and proteases in the gastrointestinal tract generally precludes infection by the oral route. [93]

VECTOR

Colour Plate:2; ***Aedes aegypti***

The vector for dengue virus is *Aedes* mosquito, which is not affected by the disease, although an infected mosquito may infect others. [57]

Aedes mosquitoes are easily distinguished by white stripes on a black body, therefore

referred to as “Tiger mosquitoes”. *Aedes aegypti* is widely distributed in India. [68]

Of the three *A*edes mosquitoes, ie*, Aedes aegypti, Aedes albopictus* and *Aedes vittatus*, that are commonly collected in TamilNadu, *Aedes aegypti* is found to be the most prevalent species. [92]

Dengue fever Antigens have been detected in *Aedes aegypti* mosquito on several occassions including certain rural areas and *Aedes aegypti* has been proved to be the primary vector of dengue. [100]

Feeding attempts may occur several times a day over the insects’ lifetime of one to four weeks. Adult mosquito shelters indoors and bite during one to two hour intervals in the morning and later afternoon. In areas with endemic transmission, one of every twenty hours may contain an infected mosquito. [41]

Vertical transmission of DV has also been shown in *Aedes aegypti* which reveals that the virus may be maintained in mosquito even during inter-epidemic periods. [94]

TRANSMISSION

Dengue viruses are transmitted to humans through the bites of infective female *Aedes* mosquito.[10]

The period of viraemia during which humans are infectious for blood feeding adult female vectors is 3 to 5 days. Humans may sustain high viraemias with one report

documenting a level of 8.3 $\log^{10}$ units per ml.[93]

After blood feeding, an extrinsic incubation period of 10 to 14 days must elapse before *Aedes aegypti* can transmit the virus upon refeeding.In rural areas and in some parts of the world *Aedes albopictus* plays a secondary role in inter-human transmission of dengue.[28]

Infected female mosquitoes may also transmit the virus to their offsprings by transovarian (via the eggs) transmission, but the role of this in sustaining transmission of virus to humans has not yet been delineated.[10] Humans are the main amplifying hosts of the virus, although studies have shown that in some parts of the world, monkeys may become infected and perhaps serve as a source of virus for uninfected mosquitoes.[10]

Epidemics of dengue peak in September to January period when an *Aedes aegypti* larval house index of more than 40% is recorded. [86] Infections can be transmitted by accidental needle stick injury too. Therefore the high incidence of infection in endemic areas suggests the possibility that, transfusion associated cases could occur. [76]

PATHOGENESIS AND IMMUNOLOGICAL REACTION

Most dengue virus infections are subclinical. Self-limited dengue fever is the usual outcome of infection but an immuno-pathogenic response in some patients, usually in the setting of heterologous immunity, produces a syndrome of dengue hemorrhagic fever. [24]

After an infectious mosquito bite, the virus replicates in local lymph nodes and within 2 to 3 days disseminates via the blood to various tissues. Virus circulates in the blood typically for 5 days in infected monocytes / macrophages, to a lesser extent and to lesser degree in B

cells and T cells. It also replicates in skin, reactive spleen lymphoid cells, and macrophages. [106]

Viral antigen can be demonstrated more widely in liver kupffer cells, renal tubular cells and alveolar macrophages and endothelia. The **malaise and flu-like symptoms** that typify dengue probably reflect patients' cytokine response. However **myalgia**, a cardinal feature of the illness may also indicate pathological changes in muscle typified by a moderate perivascular mononuclear infiltrate with lipid accumulation. [51]

Musculoskeletal pain (break-bone fever) could reflect viral infection of bone marrow elements, including mobile macrophages and dendritic cells (CD11b/CD18) and relatively non motile adventitial reticular cells.[31]

Histopathologic examination of skin from patients with **rash** discloses a minor degree of lymphocytic dermal vasculitis and variably, viral antigen. Elevated hepatic transaminase concentration have been reported in most cases of dengue with the aspartate aminotransferase (AST) level initially higher than that of alanine aminotransferase (ALT) and levels higher in DHF, compared with dengue fever. [43,34]

Shock in dengue shock syndrome occurs after the sudden extravasation of plasma into extravascular sites including pleural and abdominal cavities, usually with the defervescence of fever [55,22].The extensive increase in vascular permeability is associated with immune activation, as manifested by increased levels of plasma soluble Tumour necrosis factor receptor (sTNFR), Interlukin (IL)-8, Interferon(IF) gamma and other mediators and local endothelial production of IL-8, RANTES (Regulated on activation, normal T expressed and secreted)with apoptotic endothelial cell death. [79] In addition, immune complex formation

activates the complement system, with increase in C3a and C5a levels of IL-6 and intercellular adhesion molecule -1 are depressed in parallel with hypoalbuminemia and the general loss of serum proteins. Reduced cardiac output may contribute further to shock. [33]

The hemorrhagic diathesis which is not well understood might be due to a combination of cytokine action and vascular injury, viral antibodies binding to patients or cross reacting with plasminogen and other clotting factors, reduced platelet function and survival, and a mild consumptive coagulopathy.[38]

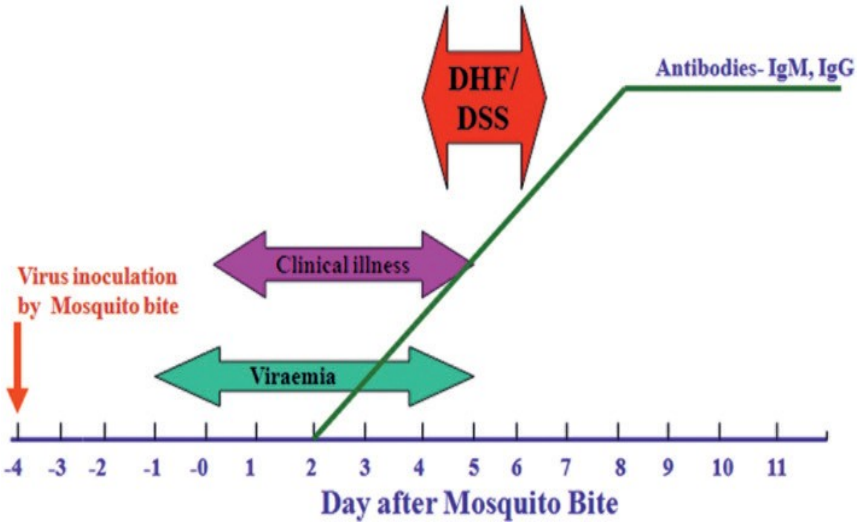
Increased frequency of dengue hemorrhagic fever in secondary dengue viral infection has suggested a role for heterologous antibodies in enhancing viral uptake and replication in Fc receptor- bearing cells (antibody mediated immune enhancement). [23] Increased levels of the TNF alpha, soluble CD8 and soluble IL-2 are higher in patients with dengue hemorrhagic fever than in dengue fever, which indicates an activation of cross reactive memory of CD4 and CD8 T- cells in response to a second infection. [57]

The resulting production of IL-2, interferon gamma and other lymphokines is reinforced by increased abundance of infected target cells resulting from interferon gamma mediated up-regulation of FC receptors and *flaviviral* induced expression of MHC type I and II molecules that further activate T-lymphocytes. [59]

Activated infected monocytes and endothelia produce and release with their lysis TNF alpha, IL-1, Platelet activating factor(PAF), IL-8 and RANTES, which act in synergy, with lymphokines, histamine and viral immune complex induced C3a and C5a to produce the temporary vascular endothelial dysfunction that leads to plasma leakage.

Illness after infection with 2 serotypes (i.e., a third bout of dengue) occurs infrequently and illness after three infections virtually never. Repeated episodes of dengue hemorrhagic fever have been recognized rarely, presumably because immune factors that promote immunopathologic responses are outweighed by immune responses that clear the infections. [75]

ANTIBODY RESPONSE



Colour plate:3; (Sequence of events during dengue infections following the bite of infected mosquito)[6]

Anti-dengue virus IgM antibody is produced transiently during primary and secondary infections.In patients with primary dengue virus infections , IgM antibodies develop rapidly and are detectable on days 3 to 5 of illness in half of the hospitalized patients. Studies of the dynamic antibody response showed that anti-dengue virus IgM levels peak at about 2 weeks postinfection and then decline to undetectable levels over 2 to 3 months . Anti-dengue virus

IgG appears shortly afterwards [20]. In contrast to primary infection, secondary infection with dengue virus results in the earlier appearance of high titres of cross-reactive IgG antibodies before or simultaneously with the IgM responses. [105]

Antibodies produced during dengue infection provides short lived protection against infection with a heterologous serotype of dengue virus. Neutralizing antibody levels correlate with protection against dengue virus. The presence of measurable levels of dengue antibody is generally protective, with the exception of low levels of cross-reacting antibodies induced by a virus of different serotype than the infecting type. In this situation, the antibody can conceivably enhance virus replication and the severity of disease manifestations (according to the immune enhancement theory of dengue pathogenesis). [9]

Retrospective studies have determined the presence of neutralizing anti-dengue antibodies in samples of serum from persons affected 40 or more years previously. [65,52,89,19].

Acute primary dengue virus infection is defined as an IgM positive and IgG negative result, and acute secondary dengue virus infection is defined as an IgM and IgG positive or IgM negative and IgG positive result. [87]

Serological tests for the identification of dengue infection rely on the detection of IgM antibodies during the acute phase of infection, either a fourfold rise in antibody titre in paired serum collections, or a single serum with a positive result in an IgM antibody capture ELISA. [105,21]

CLINICAL FEATURES

Classical dengue fever is an acute febrile disease with headaches, musculoskeletal pains and rash, but the severity of illness and clinical manifestations vary with age.

Infection is often asymptomatic or nonspecific, consisting of fever, malaise pharyngeal infection, upper respiratory symptoms and rash, particularly in children. [13]

In severe illness after incubation period of four to seven days, fever often with chills, severe frontal headache and retro orbital pain develops abruptly with a rapid progression to prostration, severe musculoskeletal sand lumbar back pain and abdominal tenderness.

Anorexia, nausea, vomiting, hyperaesthesia of skin and dysgeusia are common complaints. Initially the skin appears flushed, but in three to four days and with the lysis of fever an indistinct macular and sometimes scarlatiform rash develops sparing the palms and soles. As the rash fades or desquamates, localized clusters of petechiae on the extensor surfaces of limbs may remain. [75]

A second episode of fever and symptoms may ensue (“saddle back” pattern). Recovery may be followed by a prolonged period of listlessness, easy fatiguability, and even depression. Minor bleeding from mucosal surfaces is not uncommon and gastrointestinal hemorrhage and hemoptysis can occur. Hepatitis can also frequently complicate dengue fever. [95]

The clinical features of DHF-DSS are hemorrhagic phenomena and hypovolemic shock caused by increased vascular permeability and plasma leakage. With the defervesence of fever 2 to 7 days later, reduced perfusions and early signs of shock are manifested by central cyanosis, restlessness, diaphoresis and cool clammy skin and extremities. Abdominal pain is

the common complaint. In benign cases, BP and pulse may be maintained, but a rapid and weak pulse, narrowing of pulse pressure to less than 20 mm Hg and in most extreme cases an unobtainable blood pressure establish the shock syndrome. [75]

The platelet count declines and petechiae appear in wide spread distribution with ecchymoses. Bleeding occurs at mucosal surfaces from gastrointestinal tract and at many puncture sites. Liver is enlarged in up to 75% of cases. Pleural effusion can be detected in more than 80% of cases, which in combination with elevated hematocrit and hypoalbuminemia , reflects hemo-concentration. [75]

The presence of pleural and peritoneal effusions is associated with severe disease. Acute respiratory distress syndrome may develop with capillary alveolar leakage. In untreated patients, hypoperfusion complicated by myocardial dysfunction and reduced ejection fraction results in metabolic acidosis and organ failure. [75]

The unusual manifestations of dengue fever are dengue myocarditis, encephalopathy, encephalitis, intracranial bleed, acute fulminant hepatic failure, persistant thrombocytopenia.[11]

LABORATORY DIAGNOSIS:

Lab diagnosis of Dengue infection can be made by the detection of specific virus, viral antigen, genomic sequence and / or antibodies. [17,20,21,97]. At present, the three basic methods used by most laboratories for the diagnosis of dengue infections are viral isolation and characterization, detection of the genomic sequence by a nucleic acid amplification technology assay, and detection of dengue virus-specific antibodies. [105]

Other common laboratory findings include pancytopenia, neutropenia, increased hemoconcentration, thrombocytopenia and prolonged bleeding time. [99]

VIRUS ISOLATION AND CHARACTERISATION

For virus detection, virus isolation by cell culture and from mosquitoes remains the "gold standard", although it has gradually been replaced by the Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) method for rapid diagnosis. The isolation of viruses from clinical samples can be conveniently carried out with cultured mosquito cells, such as: AP-61, Tra-284, C6/36, AP64, CLA-1 cell lines or mammalian cells, such as: LLCMK2, Vero, BHK21 cell lines.[20]

Because of its higher sensitivity, the mosquito inoculation technique is still the method of choice for attempting dengue virus isolation from deceased patients with fatal cases or patients with severe haemorrhagic disease. [45,78]. *Aedes albopictus* [16,78] and *Toxorhynchites spendens*. [104] have been shown to be useful for dengue virus recovery. At present, virus isolation with the C6/36 cell line with acute phase serum or plasma from patients is the method of choice for routine dengue virus isolation.

Both cytopathic effects (CPE) (rounding, refractility and cell sloughing) and plaque formation are observed in these cells. Growth in cell culture consists of a rapid adoption phase followed by an eclipse phase of approximately 10-12 hr, after which infectious virus first appears and enters a log phase of replication lasting 18-24 hrs. [93]

MOLECULAR DIAGNOSIS

The field of molecular diagnosis has changed significantly over the past decade, leading to assays that are much more reliable for the detection and characterization of various pathogens. The Polymerase Chain Reaction(PCR) can be used to amplify and detect RNA viruses by using the enzyme reverse transcriptase(RT).[2] Various RT-PCR protocols for dengue virus have been identified [20,25,46]. The two-step nested RT-PCR and single-step nested RT-PCR for detection and typing of dengue viruses are well known. [46]

These assays use the dengue virus core to premembrane regions as the target sequence for dengue virus detection. They have the advantage of detecting and differentiating the four dengue virus serotypes by analyzing the unique sizes of the amplicons in the agarose gel. [72]

More recently, the fully automatic real-time PCR assays have been used more widely instead of the conventional RT-PCR methods for detection of dengue virus in acute-phase serum samples due to its advantages like- rapidity, the ability to provide quantitative measurements, a lower contamination rate, a higher sensitivity, a higher specificity, and easy standardization. [4,47,70,37]

Therefore, real-time PCR has gradually replaced the conventional PCR as the new gold standard for the rapid diagnosis of dengue virus infections with acute-phase serum

samples. [72]

Other variations on amplification techniques, such as NASBA, are becoming increasingly popular owing to their relative simplicity and the availability of standardized kits. [97]

SEROLOGICAL DIAGNOSIS

Among the viral infections that can be diagnosed by serology, dengue virus infection is the most challenging due to its cross-reactivity to homologous and heterologous *flavivirus* antigens. However, great advances in analyzing the complicated viral antigens and antibody responses have recently been made by the development of various methods that target different structural and non-structural proteins for sero-diagnosis and sero-epidemiological studies of dengue virus infection. [72]

ANTIGEN DETECTION

Recent studies have shown that ELISA and Dot-blot assays directed to the E/M antigen and the NS1 antigens in the form of an immune complex could be detected in the acute phase sera of both patients with primary dengue virus infection and patients with secondary infection. [37]

The *Flavivirus* NS1 is a 46-50 Kilodalton glycoprotein which is expressed in both membrane-associated (mNS1) and secreted (sNS1) forms and possesses both group-specific and type-specific determinants. The procedure of capture ELISA has been developed for detection of *flavivirus* NS1 in patient's sera. [69]

ANTIBODY DETECTION

Several methods have been described for the serological detection of dengue virus-specific antibodies, including ;

- Haemagglutination inhibition (HI) test. [8]
- Neutralization test. [80]
- Indirect immunofluorescent-antibody test.[98]
- Enzyme-linked immunosorbent assay (ELISA)[3]
- Complement fixation test.[15]
- Dot blotting [5]
- Western blotting [41]
- Rapid immunochromatography test [72]

Among these, capture IgM and/or IgG ELISA, and the HI test are the most commonly used serological techniques for the routine diagnosis of dengue virus infections, as they are simple and allow large number of samples to be tested. [97]

IgM and IgG ELISA have replaced the HAI assay because it has the potential to be automated and thus can accommodate a large number of samples. In addition, no processing of the serum is required and only a few microlitres of the sample are needed [21]. Antigens prepared in mouse brain or in tissue culture can be used. Several formats of immunoenzymatic assays for the detection of anti-arbovirus antibody have been described, including indirect , capture IgG , the inhibition method, and double antibody sandwich ELISA.

64,53

The presence of IgM antibodies to dengue virus in the absence of IgG antibodies indicates a primary infection, whereas when IgG antibody titres are higher than those of IgM, the presence of a secondary dengue infection is established. [10]

DENGUE IgM & IgG CAPTURE ELISA

Serum IgM/IgG antibodies, when present, combine with Anti-human IgM/IgG antibodies attached to the polystyrene surface of the microtitre plate. A concentrated pool of dengue 1-4 antigens is diluted to the correct working volume, with antigen diluent. The antigens are produced using an insect cell expression system and immunopurified utilizing a specific monoclonal antibody. An equal volume of the Horse Raddish Peroxidase (HRP)-conjugated monoclonal antibody is added to the diluted antigen, which allows the formation of Antigen-MAb (Monoclonal Antibodies) complexes. Residual serum is removed from the assay plate by washing and complexed antigen-MAb is added to the assay plate. After incubation, the microwells are washed and a colourless substrate system, tetramethylbenzidine/ hydrogen peroxide (TMB/ H_2O_2) is added.

The substrate is then hydrolysed by the enzyme and the chromogen changes to a blue colour. After stopping the reaction with acid, the TMB becomes yellow. Colour development is indicative of the presence of anti-dengue antibodies in the test sample.

RAPID DIAGNOSTIC TESTS

Lateral flow tests for dengue antibodies

Lateral flow tests for antibodies to dengue provide the same information as ELISA. Tests using recombinant viral envelope glycoproteins of dengue viruses 1, 2, 3 and 4, respectively, are being widely available as commercial kits. Although lateral flow tests for dengue may have low sensitivity than ELISAs, they are true rapid tests and have several other advantages like, ease of performance, speed, high stability with easy differentiation between primary and secondary infection using a single dilution of serum.

In Dengue Duo Cassette Rapid test by lateral flow assay, IgM & IgG are determined here simultaneously using a single addition of serum, plasma or whole blood. Therefore, differentiation between primary and secondary infection can be made through single application of sample rather than a series of dilutions as needed in Haemagglutination Inhibition (HAI) assay.

ANIMAL INOCULATION

All four dengue viruses have been successfully isolated in African green monkey kidney (Vero)cells or 1-3 days old baby mice using a soup prepared from *Ae. Aegypti*. Suckling mice are important as it is generally not possible to detect the virus in other animal host body (eg. Mosquitoes, ticks) when in low quantity. Mice are inoculated intracranially with classified suspensions of clinical specimens or macerated arthropod pools or animal tissues.

TREATMENT

There is no specific treatment for DF. However careful clinical management frequently saves the lives of DHF patients. With appropriate intensive supportive therapy, mortality may be reduced to < 1%. Maintenance of the circulating fluid volume is the central feature of DHF case management.[7]

The management of DF is supportive with bed rest, adequate fluid intake and control of fever and pain with antipyretics and analgesics. For the more severe manifestation of DV infection, appropriate management requires early recognition and rapid IV fluid replacement.[35] The hematocrit should be measured frequently.[93] In severe cases blood transfusions may be required.

On average, DHF case fatality rates are about 5%.[103] Case fatality rates can be as high as 20-40% in DHF/DSS, but can be reduced with early diagnosis, proper case management and using fluid replacement therapy.

DENGUE VACCINE

There is no vaccine for DENV/ DHF although significant progress has been made in developing both live attenuated vaccine candidates and second-generation recombinant candidate vaccines using infectious clone technology in recent years.[96]

There are three major concerns in the development of dengue vaccine. Firstly, is the possibility that it could lead to antibody- dependent enhancement of infection and thus produce DHF/ DSS. Candidate vaccines based on live attenuated viruses should therefore

contain all four serotypes to give comprehensive protection without adverse side effects. Another concern is that possibility of virus evolution through genome recombination. The third concern is that the vaccine may produce adverse reactions, for example, recently a tetravalent live attenuated vaccine was tested in human volunteers and in children, Phase I and Phase II trials have shown mildly adverse reactions with monovalent vaccines, but more frequent and significantly more severe reactions with the tetravalent vaccine. [58]

The present lack of a successful vaccine against the dengue virus, causes prevention methods to be approached.

PREVENTION

A multi-sectoral, multifaceted and comprehensive response will be required to meet the challenges of frequently occurring outbreaks. Disease surveillance, training of health care providers in medical and paramedical schools and strengthening health infrastructure has to be implemented through innovative, client-friendly approaches throughout the year on a regular and sustainable basis. [86]

The WHO guidelines [103] for prevention of dengue are that all control efforts should be directed against mosquitoes. It is important to take control measures to eliminate the mosquitoes and their breeding places. Efforts should be intensified before the transmission season (during and after the rainy season) and during epidemics.

Aims of the Study

AIMS OF THE STUDY

- To study the incidence of dengue cases among patients with fever in a tertiary care hospital.
- To determine the seropositivity of Dengue cases.
- To categorise dengue cases as dengue fever, dengue haemorrhagic fever and dengue shock syndrome , according to WHO guidelines.
- To evaluate the proportion of primary and secondary dengue infections.
- To determine the serotype of dengue virus in dengue positive cases in the early febrile period.

Materials & Methods

MATERIALS AND METHODS

STUDY PERIOD

This Cross-sectional study was done from October 2006 to October 2007.

SAMPLE

Blood samples from 250 patients with clinical features suggestive of dengue, were included in this study. The samples were collected aseptically and serum was separated by centrifugation technique and stored at -70˚C.

INCLUSION CRITERIA

The clinical basis for diagnosing the patients as having dengue fever was based on standard criteria like presentation of febrile illness of 2-7 days duration, with features like headache, myalgia, arthralgia, rash, haemorrhagic manifestations and leucopenia. [103, 82]

EXCLUSION CRITERIA

Patients with clinical evidence of urinary tract infection, pneumonia, abscess or any other apparent cause of fever were excluded. [44]

SOURCE OF SAMPLE

The samples were received from fever clinic and from in-patients with features suggestive of dengue, Madras Medical College and General Hospital, Chennai-3.

ETHICAL CONSIDERATIONS

Written consent to participate in the study was obtained from the subjects or their guardians after the full explanation of the study was provided to them. This study was

reviewed and approved by Institutional Ethical Committee, Madras Medical college & General Hospital, Chennai-3. All data were handled confidentially and anonymously.

STATISTICAL ANALYSIS

The proportional data of this cross-sectional study was tested using Pearson's Chi-Square (X^2)analysis test, Two sample binomial proportion test, Statistical analyses were carried out using Statistical Package for Social Sciences(SPSS) and Epi-Info softwares.

METHODS

The samples were subjected to **PANBIO** Rapid Duo Cassette method, IgMELISA & IgG ELISA at Institute of Microbiology, Madras Medical College & General Hospital, Chennai. Single-step nested RT- PCR was done for 28 samples at Christian Medical College, Vellore.

1. RAPID DENGUE DUO CASSETTE METHOD

The cassette contains a square well for addition of buffer solution, a circular well for serum sample and a lateral flow membrane with colloidal gold complexes containing recombinant dengue 1-4 antigens and a control.

Principle of the test

If dengue specific IgM & IgG antibodies are present in the patients sample, they bind to Anti-human IgM or IgG antibodies immobilized in 2 lines across the cassette membrane. Colloidal gold complexes containing recombinant dengue 1-4 antigens are captured by the bound patients' IgM or IgG to give visible pink lines. A control is included to indicate that the assay has been performed correctly.

Procedure

1. 10µl of whole blood, serum or plasma is added to the circular well in the cassette using a micropipette.
2. The sample is allowed to absorb entirely into the specimen pad in the circular well.
3. The buffer (Phosphate buffer saline) bottle is held vertically and 1cm above the square well, adjacent to the circular well in the cassette and 2 drops of buffer is added.
4. The result is read exactly 15 min after adding the buffer to the cassette.
5. Any trace of a pink line in the test area indicates a positive result.
6. Any results read after 15 min should be considered invalid and repeated.
    - Serological sensitivity of the test - 96.3%
    - Serological specificity of the test - 95%

Interpretation of results

Interpretation should be based on the combined results of the IgG and IgM test lines.

C- Control line

M- IgM test line

G- IgG test line

Primary infection

- Pink bands appear in the IgM & control regions
- The test is positive for IgM antibodies and is suggestive of primary dengue infection.

Secondary infection

- (1) Pink bands appear in IgM, IgG and control regions.

  The test is positive for IgM & IgG antibodies and is suggestive of secondary dengue infection.

- (2) Pink band appears in IgG and control regions.

  The test is positive for IgG antibodies and is suggestive of secondary dengue infection.

Negative

- A pink band appears in the control region only.
- No detectable IgG or IgM antibodies to dengue.

Invalid

- No pink band appears in control region.
- The test is invalid.

2.DENGUE ANTIBODY ELISA

REQUIREMENTS

1. Anti-human IgM / IgG coated microwells (Assay plate)
2. Dengue 1-4 antigens (Recombinant)
3. Wash buffer concentrate-20X concentrate of phosphate buffered saline (PBS) ,pH 7.2-7.6 with Tween 20 and 0.1% proclin as preservative.
4. Serum diluent-Tris buffered saline with preservatives and additives.
5. Antigen diluent- PBS with preservative and 0.005% gentamycin.
6. Horse Raddish Peroxidase(HRP) conjugated Monoclonal Antibody Tracer

7. Tetramethyl benzidine (TMB)- 3,3',5,5'-the substrate, tetramethyl benzidine, hydrogen peroxide in a citric-acid citrate buffer (pH 3.5-3.8)
8. Positive control serum,Negative control serum, and cut-off calibrator - Human serum with 0.1 % sodium azide and 0.005% gentamycin sulphate.
9. Stop solution-1Mole Phosphoric acid.

DENGUE IgM CAPTURE ELISA

PROCEDURE

Serum predilution

1. The microwells are inserted into the strip holder. 5 microwells are required for positive control (PC), negative control(NC) and cutt-off calibrator (CO) in triplicate.
2. The PC,NC & CO & patient samples are diluted using suitable test tubes or microtitre plate.
3. 1000 µl or 1ml of serum diluent is added to 10µl of serum and mixed well.

Elisa procedure

1. Antigen is diluted 1/250 using the antigen diluent. ie, 10µl of antigen + 2.5 ml of antigen diluent. A volume of 0.5 ml of diluted antigen is required per strip.
2. Required volume of diluted antigen is mixed with equal volume of MAb tracer (Horse Raddish Peroxidase conjugated Monoclonal antibody tracer) in a test tube and kept at room temperature (20- 25˚C) until required.
3. 100µl of diluted patient sample and controls (one positive control, one negative control and three cut-off calibrators) are pipetted into their respective microwells of the assay plate.

4. The plate is covered and incubated for 1 hour at 37˚C.

5. After incubation , the plate is washed 6 times with diluted wash buffer.
6. The antigen- MAb tracer solution is mixed well and 100µl is transferred to microtitre wells.
7. The plate is covered and incubated for 1 hour at 37˚ C.
8. The plates are washed 6 times with diluted wash buffer after incubation.
9. 100µl of TMB(Tetramethylbenzidine) is pipetted into each well and a blue colour develops. The plate is incubated for 10 min at room temperature.
10. At the end of 10min, 100µl of stop solution is pipetted into all wells. The blue colour will change into yellow.
11. The absorbance of each well is read at a wavelength of 450nm with a reference filter of 600-650nm, using a dual wavelength spectrophotometer.

Calculations

- The cut-off value was determined by calculating the average absorbance of the triplicate of the cut-off calibrator.
- The index value was calculated by dividing the sample absorbance by the cut-off value.
- Panbio units can be calculated by multiplying the index value by 10.

$$\text{Index value} = \frac{\text{Sample absorbance}}{\text{Cut-off value}}$$

Panbio units= Index value x 10.

Test validity:

Calibrator mean ≥ 1.5 x Negative absorbance.

$$\frac{\text{Positive control}}{\text{Cut-off}} = 1.1\text{-}6.0$$

Negative control < 0.350

Interpretation of results

Index	Panbio units	Results
<0.9	<9	Negative
0.9-1.1	9-11	Equivocal
>1.1	>11	Positive

Sensitivity of this test is 94.7%, Specificity is 100%.

3.DENGUE IgG CAPTURE ELISA

PROCEDURE

The dengue IgG ELISA is set to detect high levels of IgG present in secondary but not primary or past dengue infections. All the reagents were brought to room temperature and serum pre- dilution done as for dengue IgM capture ELISA.

Elisa procedure

1. Antigens are reconstituted with antigen reconstitution buffer. 1ml of reconstitution buffer was added to antigen and mixed.

2. Required volume of reconstituted antigen is mixed with an equal volume of MAb tracer (Horse Raddish Peroxidase conjugated Monoclonal antibody tracer) in a test tube and kept at room temperature until required.
3. Add 100µl of diluted patient sample and controls into their respective microwells of the assay plate (anti-human IgG coated microwells).
4. The plate is covered and incubated for 30 min at 37*C.
5. After incubation, the plate is washed 6 times with diluted wash buffer.

6. The antigen- MAb tracer solution is mixed well and 100µl is transfered to microtitre wells.
7. The plate is covered and incubated for 1 hour at 37* C.
8. The plates are washed 6 times with diluted wash buffer after incubation.
9. 100µl of Tetramethylbenzidine is pipetted into each well and incubated for 10 min at room temperature, a blue colour will develop.
10. At the end of 10min, 100µl of stop solution is pipetted into all wells. The blue colour will change into yellow.
11. The absorbance of each well is read at a wavelength of 450nm with a reference filter of 600-650nm, using a dual wavelength spectrophotometer.

Calculations

- The cut-off value was determined by calculating the average absorbance of the triplicate of the cut-off calibrator.

- The index value was calculated by dividing the sample absorbance by the cut-off value.
- Panbio units can be calculated by multiplying the index value by 10.

$$\text{Index value} = \frac{\text{Sample absorbance}}{\text{Cut-off value}}$$

Panbio units= Index value x 10.

Test validity:

- Calibrator mean > Negative absorbance.
- $\frac{\text{Positive control}}{\text{Cut-off}} = 1.1\text{-}6.0$
- Negative control < 0.350

Interpretation of results:

Index	Panbio units	Results
<0.9	<9	Negative
0.9-1.1	9-11	Equivocal
>1.1	>11	Positive

Sensitivity of this test is 85.7% and specificity is 100%.

4. SINGLE STEP NESTED RT-PCR USING NS3 PRIMERS

(i).Viral RNA Extraction. (Qiagen Viral RNA Extraction kit) [83,1]

Requirements:

1. QIAamp membrane (Provided in the kit)
2. Wash buffers-1 & 2 (Guanidine hydrochloride buffers, differing by concentration)
3. Elution buffer (RNase free buffer)
4. Ethanol (96-100%)
5. Carrier RNA
6. Buffer 3 (Guanidine thiocyanate)
7. 1.5ml microcentrifuge tube
8. Microcentrifuge and Vortex equipments

Principle

- The sample is first lysed under highly denaturing conditions (provided by buffer 3) (Guanidine isothiocyanate method) [44] to inactivate RNAses & to ensure isolation of intact viral RNA.
- Carrier RNA is added to buffer 3, to improve the binding of viral RNA to the QIA amp membrane.

- Bufffering conditions are then adjusted to provide optimum binding of the RNA to the QIAamp membrane, and the sample is loaded on to the Mini spin column.
- The viral RNA binds to the membrane and contaminants are efficiently washed away in 2 steps using 2 different wash buffers- 1 & 2.
- Elution is done to obtain High-quality RNA using a special RNase- free buffer, the elution buffer that contains 0.04% sodium azide.
- The purified RNA is free of protein, nucleases and other contaminants and inhibitors. The total procedure time is 20 minutes.
- Determination of viral RNA yield is difficult, because, they are normally less than 1µg and therefore difficult to determine photometrically. Therefore, Quantitative RT-PCR is done to determine the viral RNA yield.

Viral RNA Extraction Procedure:

- 560µl of buffer 3 containing carrier RNA was pippeted into a 1.5 ml microcentrifuge tube.
- The serum sample was added to the tube and mixed by pulse- vortexing for 15 seconds.
- The mixture was incubated for 10 minutes at room temperature.
- The tube was centrifuged briefly to remove drops from the inside of the lid.
- 560µl of ethanol (96-100%) was added to the sample, and mixed by pulse-vortexing for 15 secs. After mixing, the tube was briefly centrifuged to remove drops from inside the lid.
- 630µl of solution was added to a 2ml collection tube and centrifuged for 1 min at 8000 rpm. The collection tube was placed in the spin column and the tube containing the

filtrate was discarded.

- Step 6 was repeated.
- 500µl of buffer 1(Guanidine hydrochloride) was added to the Mini spin column & centrifuged at 8000 rpm for 1 min. The Mini spin column was placed in a 2ml collection tube and the filtrate discarded.
- 500µl of buffer 2 was added, cap closed & centrifuged at full speed, at 14000 rpm for 3 minutes.
- The QIA amp mini spin column was placed in a 1.5 ml micro-centrifuge tube and the old container tube containing filtrate was discarded.
- The QIA amp spin column was opened and 60µl of elution buffer was added (to elute the viral RNA from the QIAamp mini spin column) and incubated at room temperature for 1 minute.
- After centrifugation at 8000 rpm for 1 minute, the viral RNA is ready for RT-PCR.

(ii) SINGLE-STEP NESTED RT-PCR

Principle

The single-step nested PCR reaction consists of using two primer sets directed against the same target, wherein both sets of primers are added together and an extended PCR is performed.[35]

Components of PCR

Oligonucleotides

Oligonucleotides used in this procedure were 17-23 nucleotides in length. They were used at a concentration of 10 pmol for 35 cycles of amplification.

Description of primers

Primer	5'-3' sequence	Nucleotide position	Target size(when used with DV1)	Length of the sequence
DV1	GGRACKTCAGGWTCTCC	4918-4934	-	17
DSP1	AGTTTCTTTTCCTAAACACCTCG	5067-5045	169bp	23
DSP2	CCGGYGTGCTCRGCYCTGAT	5279-5260	362bp	20
DSP3	TTAGAGTYCTTAAGCGTCTCTTG	5174-5152	265bp	23
DSP4	CCTGGTTGATGACAAAAGTCTTG	5342-5320	426bp	23

Buffer used

1X PCR buffer containing 1.5mM magnesium chloride ($MgCl_2$) was used.

Taq. DNA polymerase

Taq polymerase,derived from *Thermus aquaticus*, was used in the reaction, which carries a 3' to 5' exonuclease activity. The final concentration of Taq polymerase used was 0.5U.

Deoxynucleotide Triphosphate(dNTP's)

dNTP's was used at a saturating concentrartion of 0.2 mM .

Reverse transcriptase

Commercially available reverse transcriptase (derived from Moloney murine leukemia virus -MMLV), in a concentration of 25U was used for the procedure.

Positive control

Dengue virus serotypes 1-4 were received from National Institute of Virology (NIV),Pune and cultured in African green monkey kidney cells(Vero). The cytopathic effects of rounding and detachment from the surface was seen by fifth day after the passage. This was used as the positive control.

PCR Reaction Mixture

Components	Final concentration of reagents	Quantity of reagents
PCR buffer	$MgCl_2$ 1.5mM, Tris-HCl 50mM, KCl 75mM, pH 8.3	10µl
dNTP	0.2mM	2µl
Forward Primer	10pM	2µl
Reverse Primers	10pM	8µl (2x4)
Taq.Polymerase	0.5U	1µl
Reverse transcriptase	25U	1µl
Water		6µl
RNA extract (Template)		20µl
Total volume of each reaction		50µl

SINGLE –STEP NESTED RT-PCR PROCEDURE (Seah et al,1995)[82]

1. This method was performed in a 50µl volume comprising- sample RNA template, PCR buffer with 1.5mM $MgCl_2$, DV1 & DSP 1-4 primers 10pM each, 25U of reverse transcriptase , 0.5U of Taq polymerase ,0.2mM dNTP.
2. The mixture was subjected to RT at 50˚C for 15 minutes, followed by an initial 95˚C for 1 minute, 10 PCR cycles of denaturation at 95˚C for 0.5 minute ,annealing at 50˚C for 1 minute between each segment ; and 20-25 PCR cycles of 95˚C for 0.5 minute, 50˚C for 0.5 minute & 72˚C for 0.5 minute with a ramp temperature of 0.5 minute.

3. Amplification was carried out in Perkin Elmer Gene amp PCR system.
4. 10µl of each PCR product were resolved by electrophoresis in 2% agarose gels in TBE buffer (pH-8.0), containing 0.5µL ethidium bromide and viewed under a UV transilluminator.
5. To avoid risk of false positives, both positive and negative controls were included in the assay.
6. If positive amplification is present, bands will be seen at 169 bp, 362 bp, 265 bp and 426 bp.

Results

RESULTS

Total number of samples tested : 250

- PANBIO Rapid Duo Cassette method, IgM ELISA and IgG ELISA methods were done for the samples at Institute of Microbiology, Madras Medical college & General Hospital, Chennai-3
- Single-step nested RT-PCR was done for 28 samples at Christian Medical College, Vellore.
- Clinical data was collected for all patients.

TABLE-1:

INCIDENCE OF DENGUE FEVER

Total number of fever cases	Suspected Number Of cases	Dengue positive cases	Incidence
10113	250	90	0.88

Incidence rate=90/10113x100=**0.88%**

(0.72%-1.10%) with 95% confidence interval

Burden of disease=1 out of 112 patients.

TABLE-2

SEROPOSITIVITY OF DENGUE

Total no. of suspected Cases	Total no. of positive Cases	Percentage (%)
250	90	36

Seropositivity = 36% (Range=30-42%) with 95% Confidence Interval

TABLE- 3

AGE DISTRIBUTION OF DENGUE CASES (n=90)

Age group	Total cases	Positive cases	Percentage (%)
0-20yrs	72	31	43.05
21-40yrs	110	51	46.36
>40yrs	68	8	11.76

Age group 21-40yrs was commonly involved in both sexes

Chi Square(X^2) test = 14.06; p=0.001 (Significant)

TABLE-4

SEX DISTRIBUTION OF DENGUE CASES

Sex	Total cases	Positive cases	Percentage (%)
Males	119	51	42.85
Females	131	39	29.77
Total	250	90	

Two sample Binomial proportion test =2.15; p=0.03. (Significant)

TABLE - 5

CLINICAL PRESENTATION OF DENGUE (n=90)

S.No	Clinical Features	Number of patients	Percentage (%)
1.	Fever	90	100
2.	Myalgia / Arthralgia	64	71.1
3.	Headache	44	48.88
4.	Haemorrhagic manifestations	35	38.88
5.	Rash	25	27.77
6.	Gastro intestinal symptoms	20	22.22
7.	Hepatomegaly	15	16.66
8.	Retro-orbital pain	12	13.33

Fever was common to all cases. Other than fever, Myalgia / Arthralgia predominated the symptoms, followed by Headache.

TABLE- 6

HAEMORRHAGIC MANIFESTATIONS OF DENGUE CASES (n=35)

Symptoms	Number of cases	Percentage (%)
Bleeding gums	9	25.71
Petechiae	8	22.85
Haematemesis	5	14.28
Malena	5	14.28
Epistaxis	5	14.28
Vaginal bleeding	2	5.71
Ecchymosis	1	2.85

The common haemorrhagic manifestations seen in dengue patients were gum bleeding followed by petechiae.

TABLE- 7

SEASONAL DISTRIBUTION OF DENGUE CASES

Month / Year	Total number of suspected Cases	Total number of positive Cases	Percentage (%)

Oct'06	17	6	35.29
Nov'06	37	10	28.57
Dec'06	26	9	34.61
Jan'07	17	4	23.52
Feb'07	11	1	9.09
Mar'07	10	1	10
Apr'07	5	0	0
May'07	7	2	28.57
Jun'07	9	1	11.11
Jul'07	16	3	18.75
Aug'07	16	9	56.25
Sep'07	24	13	54.16
Oct'07	55	31	56.36
Total	250	90	

Increase in incidence of dengue cases during rainy season is clearly seen from this table

TABLE- 8

THROMBOCYTOPENIA IN DENGUE CASES(n=90)

Platelet count (lakhs/cu.mm)	Total cases	Percentage (%)
<20,000	27	30
21,000 -40,000	16	17.77
41,000-50,000	8	8.88
50,000-1 lakh	30	33.33
> 1 lakh	9	10

All dengue cases had thrombocytopenia and most cases had a platelet count of 50,000-1 lakh/cu.mm.

TABLE- 9

ANTIBODY RESULTS IN EARLY & LATE FEBRILE PERIOD (n=90)

Duration	IgM ELISA	%	IgG ELISA	%	Both Positive	%
1-5 days (n=45`)	30	66.66	3	6.66	12	26.66
6-10 days (n=29)	2	6.89	7	24.13	30	68.96
>10 days (n=16)	2	12.5	10	62.5	4	0.25
Total % of Antibody detection	IgM-37.77%		IgG-22.22%		Both-51.11%	

IgM positivity was more in the early febrile period and both IgM & IgG were common in the 6-10 day fever group.

X^2 square test = 57.69; p = 0.001 (Significant)

TABLE- 10

CATEGORISATION OF DENGUE CASES (n=90)

Category of dengue	No.of cases	Percentage (%)
Dengue fever	45	50
Dengue haemorrhagic fever	38	42.22
Dengue shock syndrome	7	7.7

More number of cases were found in the dengue fever group.

TABLE- 11

CLASSIFICATION OF DENGUE BASED ON WHO GUIDELINES[103]

(i) DENGUE FEVER (n=45)

Sl.No.	Signs&Symptoms	No.of Patients	Percentage (%)
1	Headache	26	57.77
2	Retro-orbital pain	12	26.66
3	Myalgia/Arthralgia	32	71.11
4	Rash	26	57.77
5	Haemorrhagic manifestations	9	20.00
6	Leukopenia	25	55.55

Myalgia/ Arthralgia followed by headache & rash were the common manifestations in the dengue fever group.

(ii)DENGUE HAEMORRHAGIC FEVER(n=38)

Sl.No	Signs&Symptoms	No.of Patients	Percentage (%)
1	Petechiae,Ecchymosis	9	23.68
2	Bleeding from mucosal sites	7	18.42
3	Hemetemesis/Malena	10	26.31
4	Thrombocytopenia <1 lakh >1 lakh	 36 2	 94.73 5.26
5	Evidence of plasma Leakage (>20% ↑in hematocrit, Pleural effusion, Ascites, Hypoprotenemia)	24	63.15

Thrombocytopenia was seen more in the dengue haemorrhagic fever group.

(iii) DENGUE SHOCK SYNDROME (n=7)

Sl.No	Signs&Symptoms	No.of cases	Percentage (%)
1	Rapid, weak pulse	6	85.71
2	Narrow pulse pressure	5	60
3	Hypotension	7	100
4	Cold, Clammy skin & restlessness	7	100

TABLE- 12

COMPARISION OF CLINICAL FEATURES AND LAB PARAMETERS BETWEEN DF & DHF/DSS

Variables	DF Positive / % (n=45)	DHF / DSS Positive / %(n=45)
Clinical Features		
Age group	21-40 yrs	21-40yrs
Sex	Males (64.44%)	Females (60%)
Haemorrhagic Manifestations	29(64.44%)	36(80%)
Retro-orbital Pain	12(26.66%)	4(8.88%)
Average days of stay in the hospital	4-6	5-11
Platelet transfusions	3	26
Outcome		
Deaths	-	2
Lab Findings		
Elevated SGOT(AST) (>40U/mL)	16(35%)	31(68.88%)
Elevated SGPT(ALT) (>35U/mL)	14(35%)	26(57.77%)
Elevated BT/CT	26(57.77%)	41(91.11%)
Thrombocytopenia (Platelet< 1.5lakhs/cu.mm)	38(84.44%)	43(95.55%)
Hypoproteinemia (Total protein<6 gms)	-	24(53.33%)
Elevated Haematocrit (Haematocrit>45%)	-	26(57.77%)
Leukopenia (<4000/cu.mm)	24(53.33%)	28(62.22%)

TABLE- 13

COMPARISION OF VARIOUS METHODS USED FOR DIAGNOSIS OF DENGUE

Methods	Tested	Positive	Percentage (%)
Rapid test	250	86	34.4
IgM ELISA	250	80	32
IgG ELISA	250	66	26.4

Rapid detection method was more sensitive than ELISA

for the detection of dengue from clinically suspected cases

TABLE- 14

DENGUE SEROTYPING BY SINGLE-STEP NESTED RT-PCR(n=28)

Antibody status	No. of samples tested	No. positive
IgM positive & IgG Negative	15	Nil
IgM & IgG negative	13	1

Detection of the viral genome was positive in the antibody negative group

TABLE-15

PRIMERS USED IN SINGLE-STEP NESTED RT-PCR FOR IgM & IgG NEGATIVE SAMPLES (n=13)

Primers	Number of samples tested	Number of samples Positive

DSP-1 (Dengue serotype-1)	13	1
DSP-2 (Dengue serotype-2)	13	Nil
DSP-3 (Dengue serotype-3)	13	Nil
DSP-4 (Dengue serotype-4)	13	Nil

Dengue serotype 1 was identified in a case by Single-Step Nested RT-PCR

TABLE- 16

DETAILS OF SINGLE STEP NESTED RT-PCR POSITIVE DENGUE CASE

Age/Sex	Fever duration	Antibody status	Other symptoms
19/F	2 days	IgM & IgG ELISA negative	Headache, Vomiting. Platelet-1 lakh

Both antibodies were negative in this patient with 2 days fever duration.

TABLE- 17

CATEGORISATION OF DENGUE INTO PRIMARY AND SECONDARY INFECTIONS BASED ON IgM : IgG RATIO[61] (n=90)

IgM : IgG Ratio	Number of cases	Percentage	Interpretation

≥1.78	26	28.88	Primary Dengue Infection
<1.78	64	71.11	Secondary Dengue infection

The incidence of secondary dengue infection was much higher than primary dengue infection in the present study.

The proportion of primary to secondary dengue was found to be **1:2.5**

TABLE- 18

CASE FATALITY IN DENGUE CASES

Total Dengue Cases	Number of Deaths	CFR
90	2	2.2%

Range = 1-9% (Based on Odds Ratio)

Incidence of Mortality = 1 out of 45 dengue cases.

TABLE- 19:

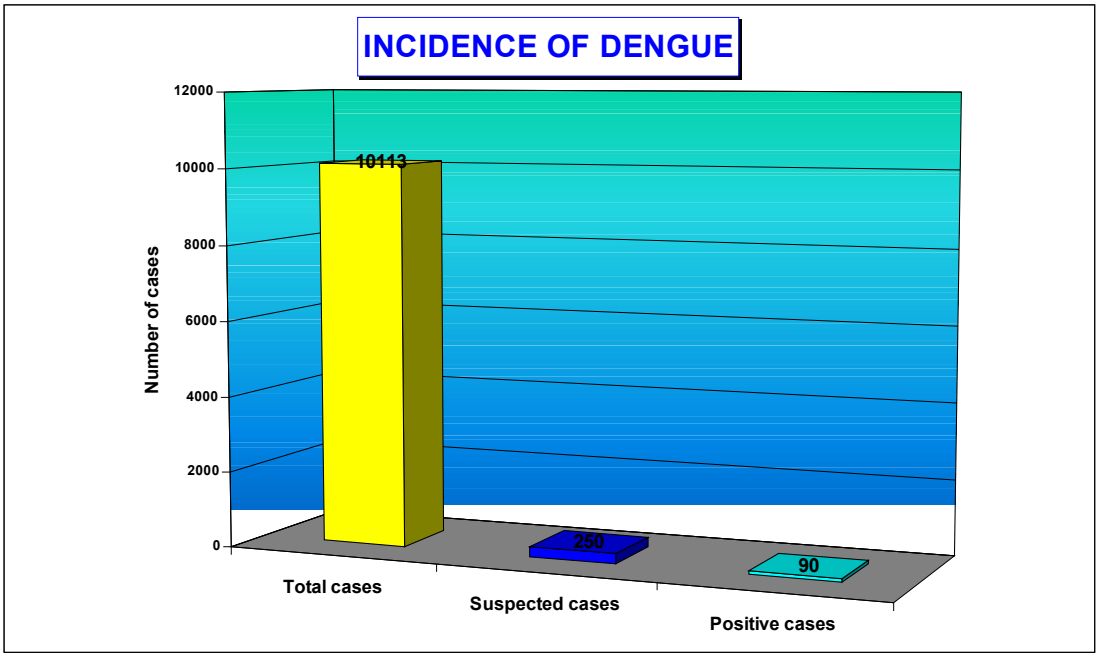
CLINICAL CLASSIFICATION OF 2 FATAL DENGUE CASES

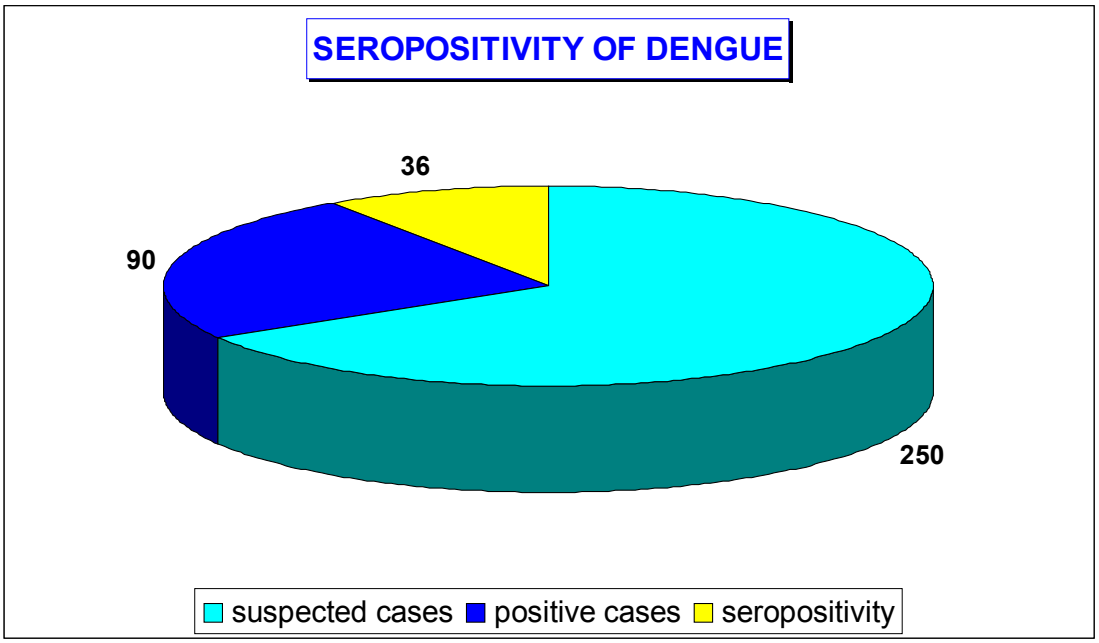
Case	Sex/ Age	Underlying Conditions	Presentation of symptoms	Classif i -cation	Hospital stay (days)	Cause of death

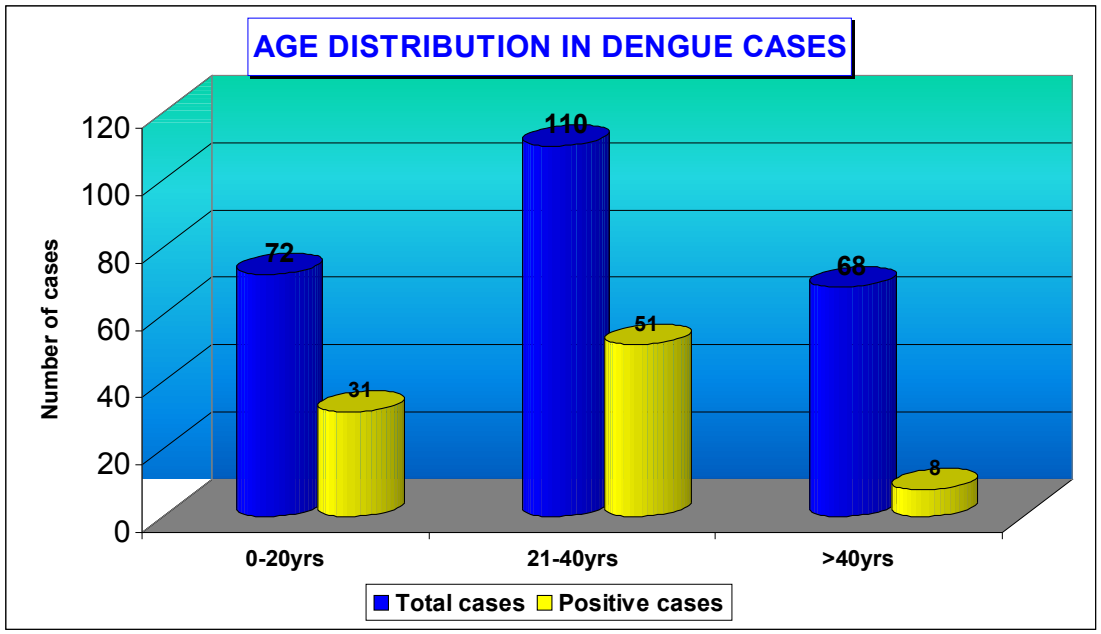
1	F/21	Nil	Fever, Hemetemesis, Renal failure, Peritoneal dialysis done	DSS	3	Profound shock, HF, ARF, brainstem dysfunction, ICH, Cardio-Pulmonary arrest.
2	F/22	Antenatal	Fever, Pallor, Rashes, Dyspnea, Peripheral cyanosis, Purpura	DSS	2	Profound shock, Cardio-pulmonary arrest.

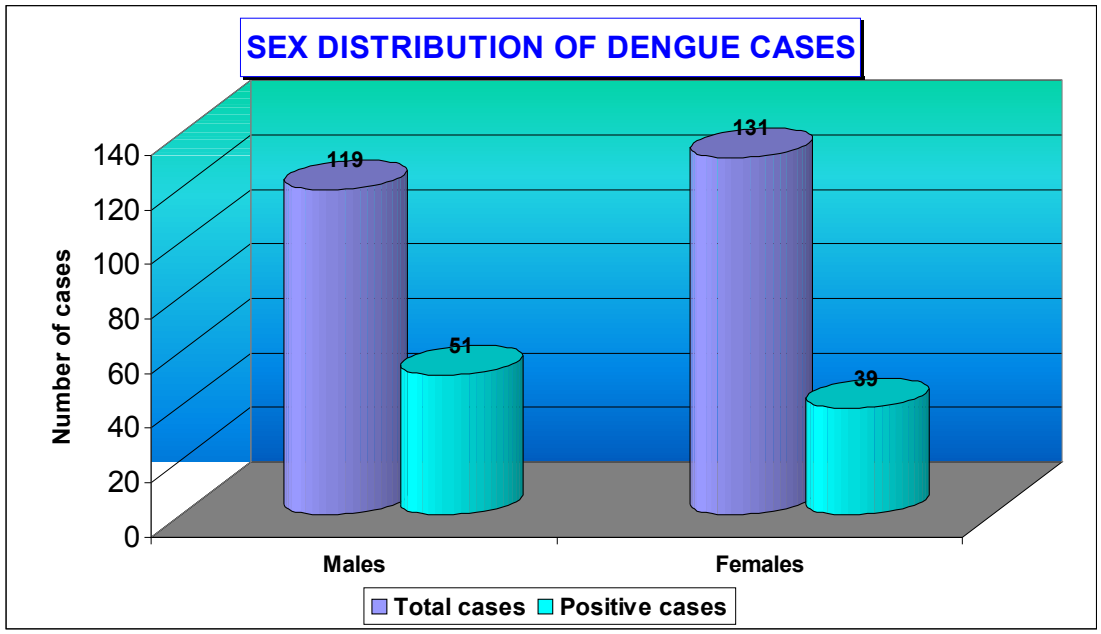
HF-Hepatic failure; ARF-Acute renal failure; ICH-Intra cranial haemorrhage

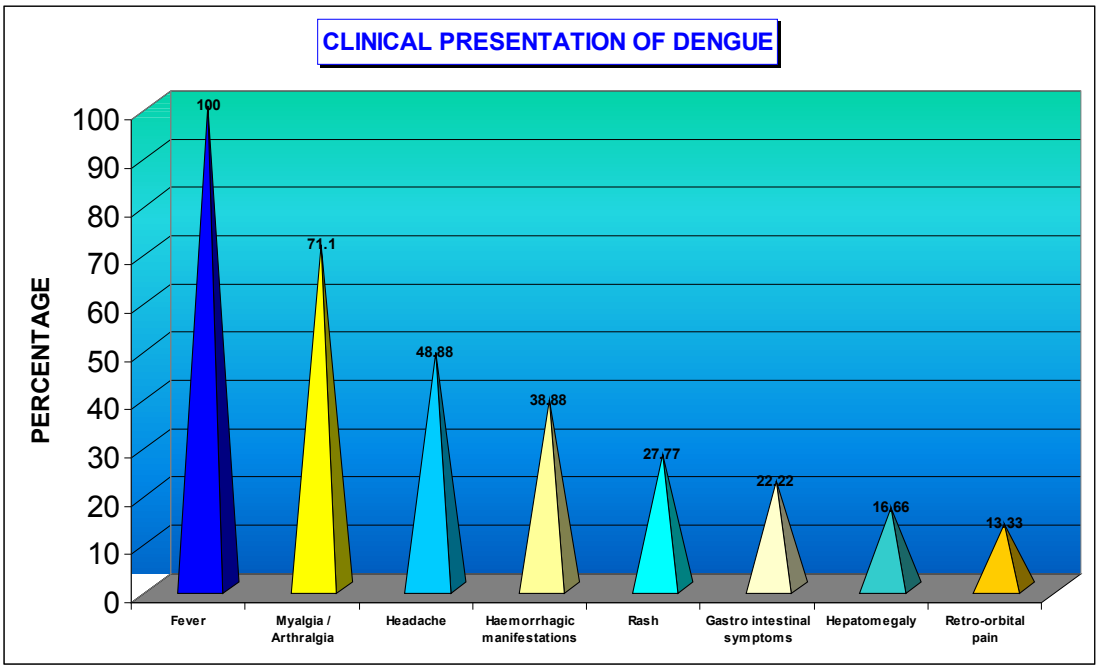
Charts

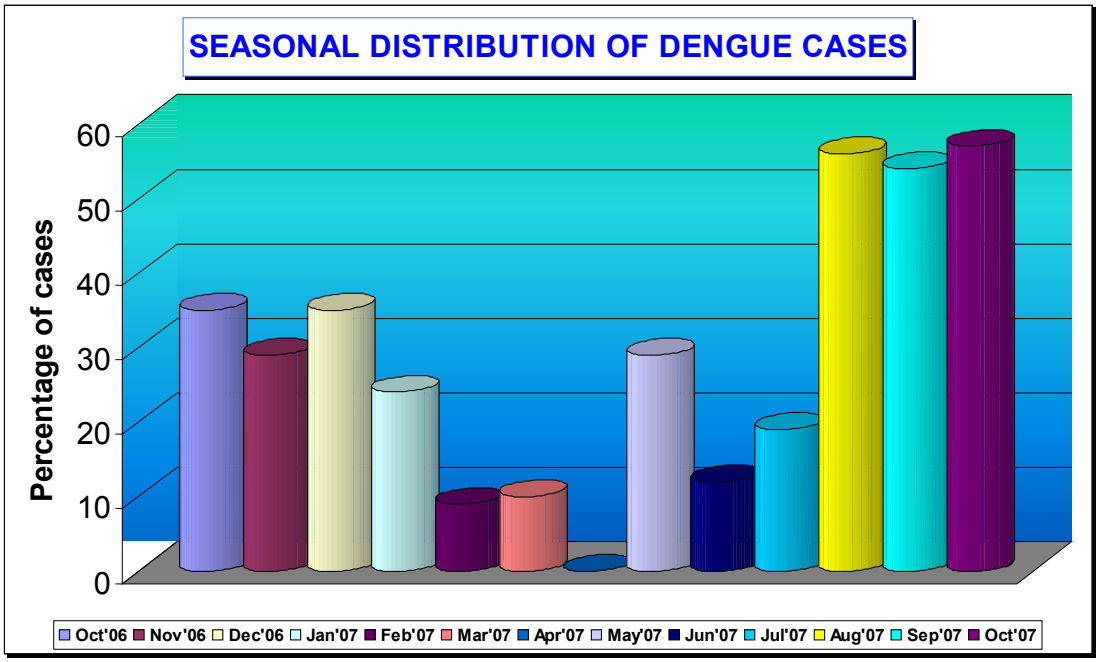

INCIDENCE OF DENGUE
12000
10000
8000
6000
4000
2000
0
Number of cases
10113
250
90
Total cases
Suspected cases
Positive cases


SEROPOSITIVITY OF DENGUE
36
90
250
suspected cases
positive cases
seropositivity

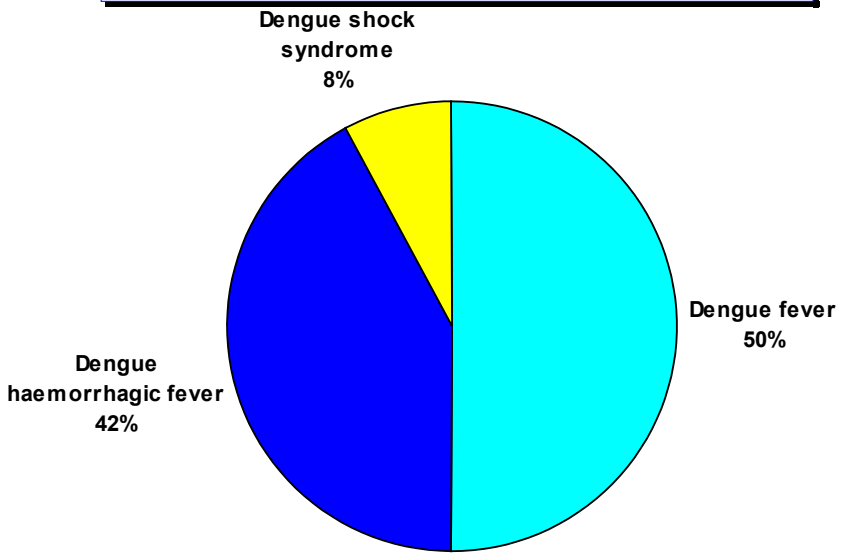

AGE DISTRIBUTION IN DENGUE CASES
Number of cases
120
100
80
60
40
20
0
72
31
110
51
68
8
0-20yrs
21-40yrs
>40yrs
Total cases
Positive cases


SEX DISTRIBUTION OF DENGUE CASES
Number of cases
140
120
100
80
60
40
20
0
119
51
131
39
Males
Females
Total cases
Positive cases

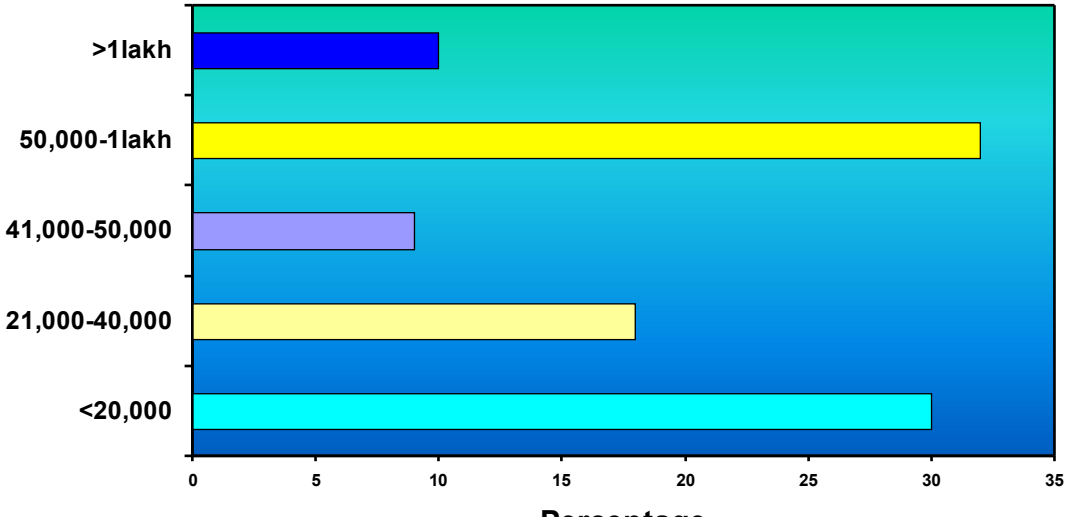

CLINICAL PRESENTATION OF DENGUE
PERCENTAGE
100
90
80
70
60
50
40
30
20
10
0
100
71.1
48.88
38.88
27.77
22.22
16.66
13.33
Fever
Myalgia / Arthralgia
Headache
Haemorrhagic manifestations
Rash
Gastro intestinal symptoms
Hepatomegaly
Retro-orbital pain

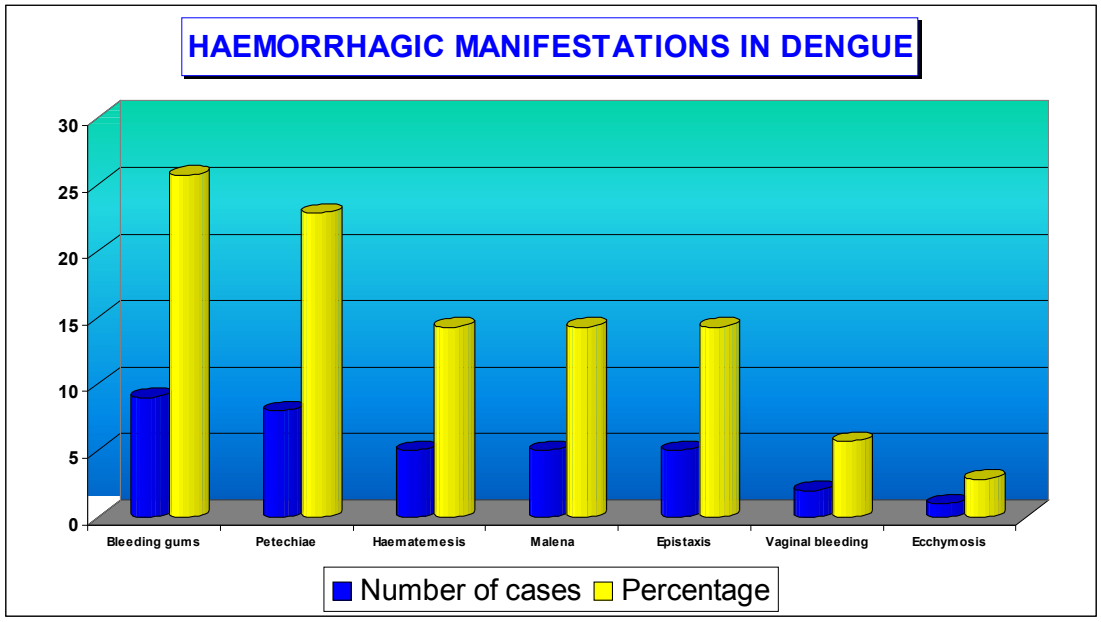

SEASONAL DISTRIBUTION OF DENGUE CASES
Percentage of cases
60
50
40
30
20
10
0
Oct'06
Nov'06
Dec'06
Jan'07
Feb'07
Mar'07
Apr'07
May'07
Jun'07
Jul'07
Aug'07
Sep'07
Oct'07

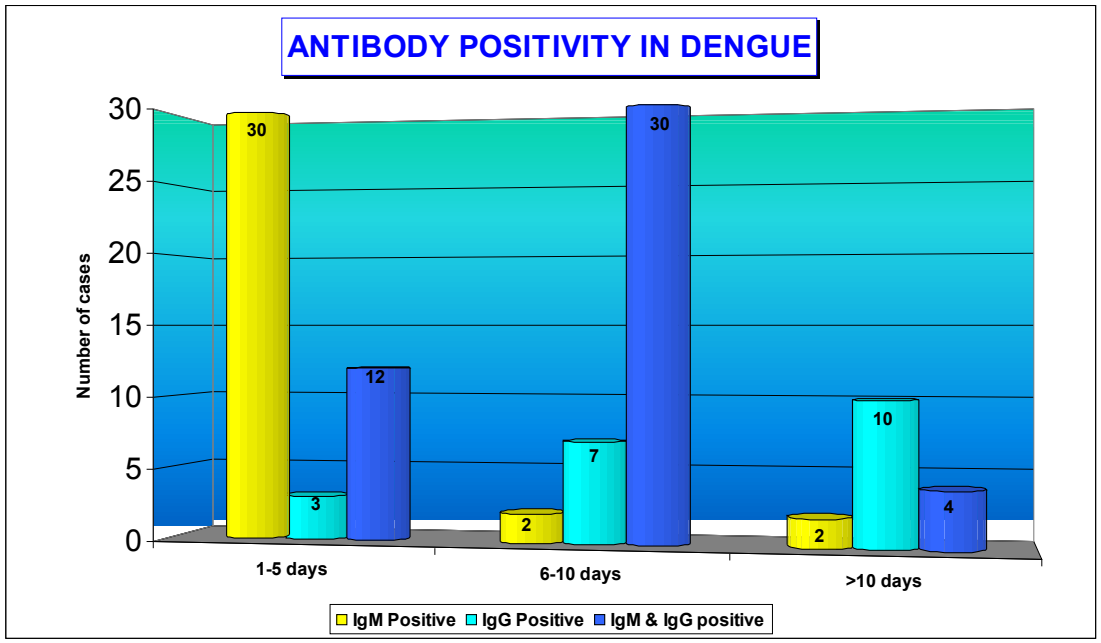
CLASSIFICATION OF DENGUE CASES

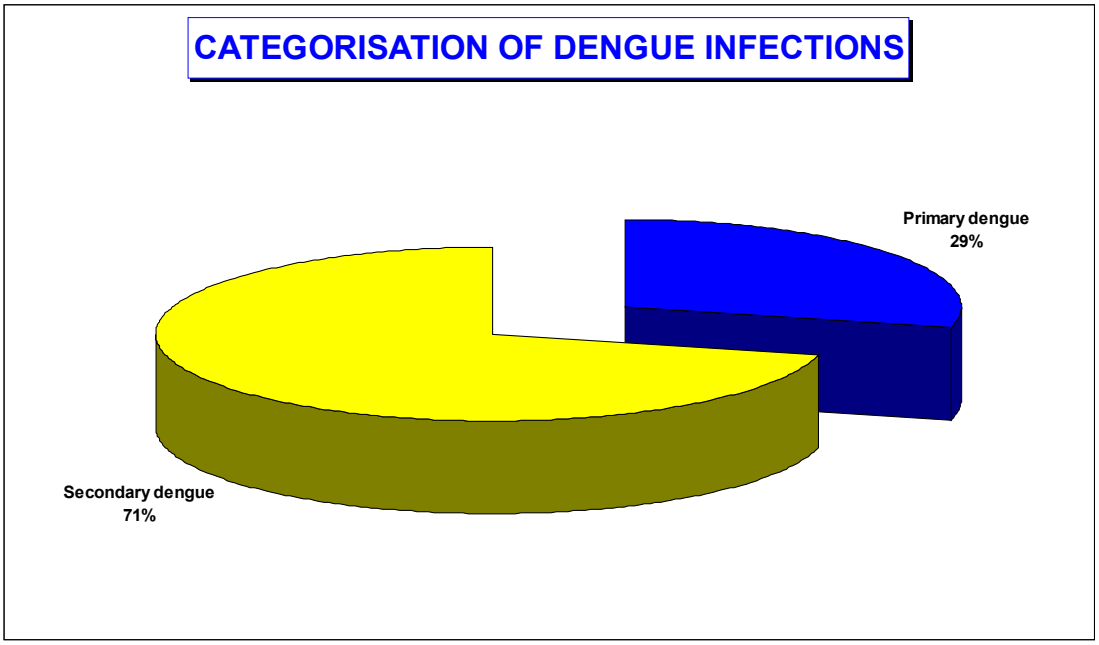


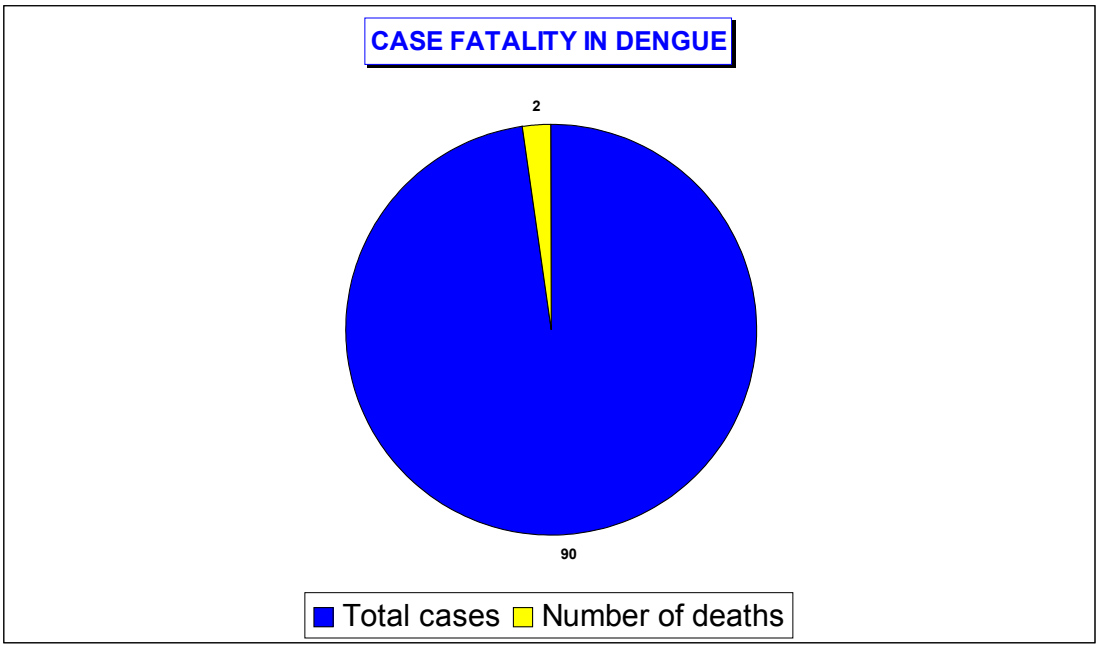
THROMBOCYTOPENIA IN DENGUE PATIENTS(lakhs/cu.mm)




HAEMORRHAGIC MANIFESTATIONS IN DENGUE
30
25
20
15
10
5
0
Bleeding gums
Petechiae
Haematemesis
Malena
Epistaxis
Vaginal bleeding
Ecchymosis
Number of cases
Percentage

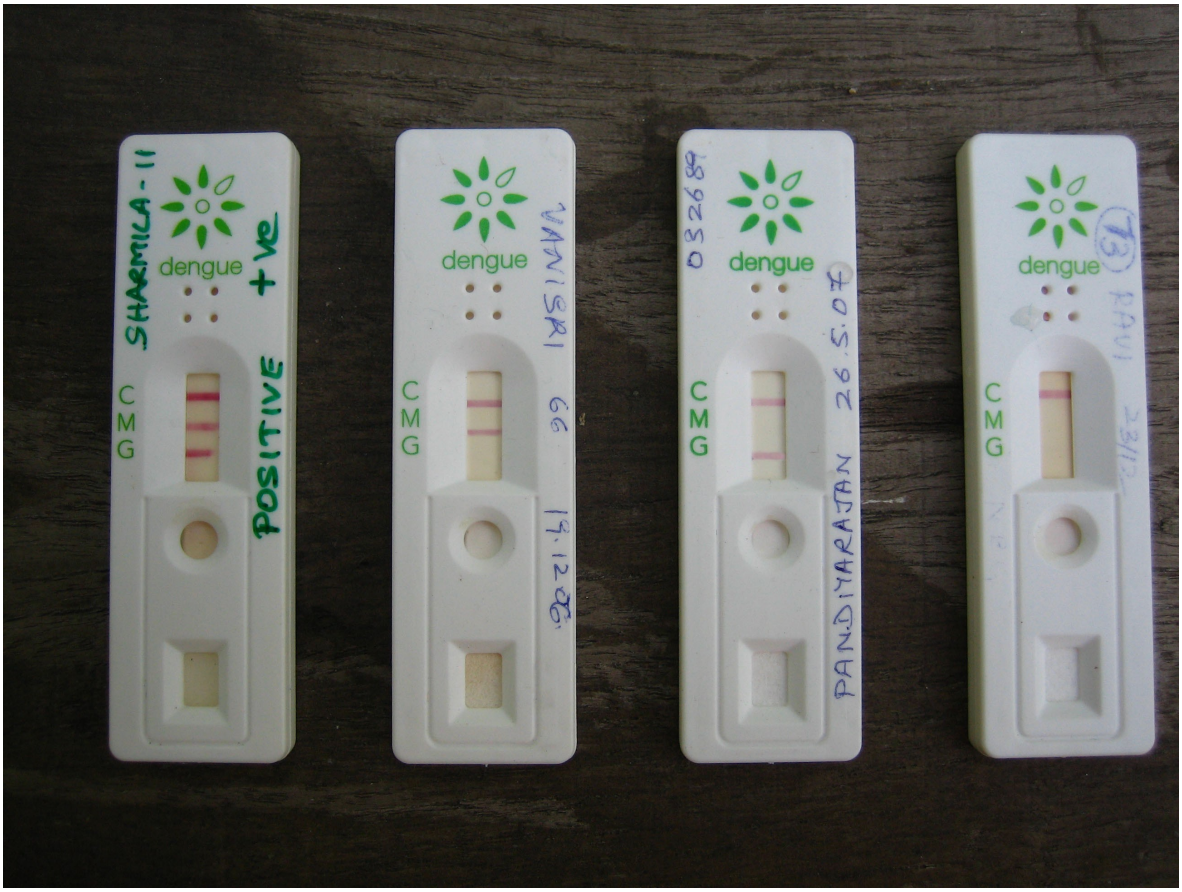

ANTIBODY POSITIVITY IN DENGUE
Number of cases
30
25
20
15
10
5
0
30
3
12
2
7
30
2
10
4
1-5 days
6-10 days
>10 days
IgM Positive
IgG Positive
IgM & IgG positive


CATEGORISATION OF DENGUE INFECTIONS
Primary dengue
29%
Secondary dengue
71%


CASE FATALITY IN DENGUE
2
90
Total cases
Number of deaths

Colour Plates

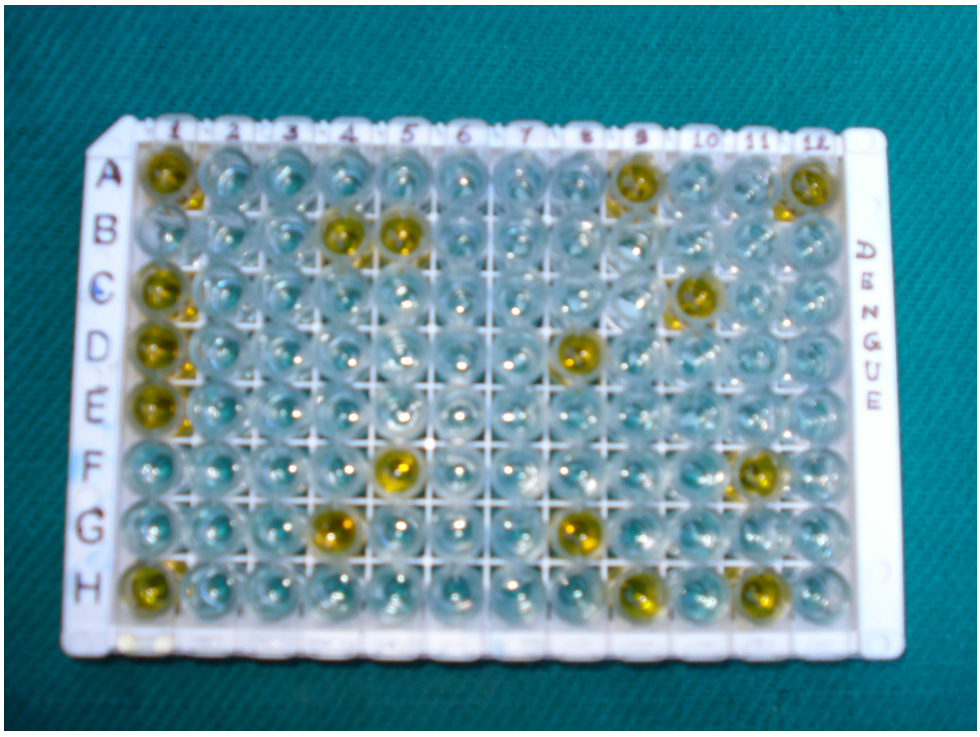
dengue rapid duo cassette



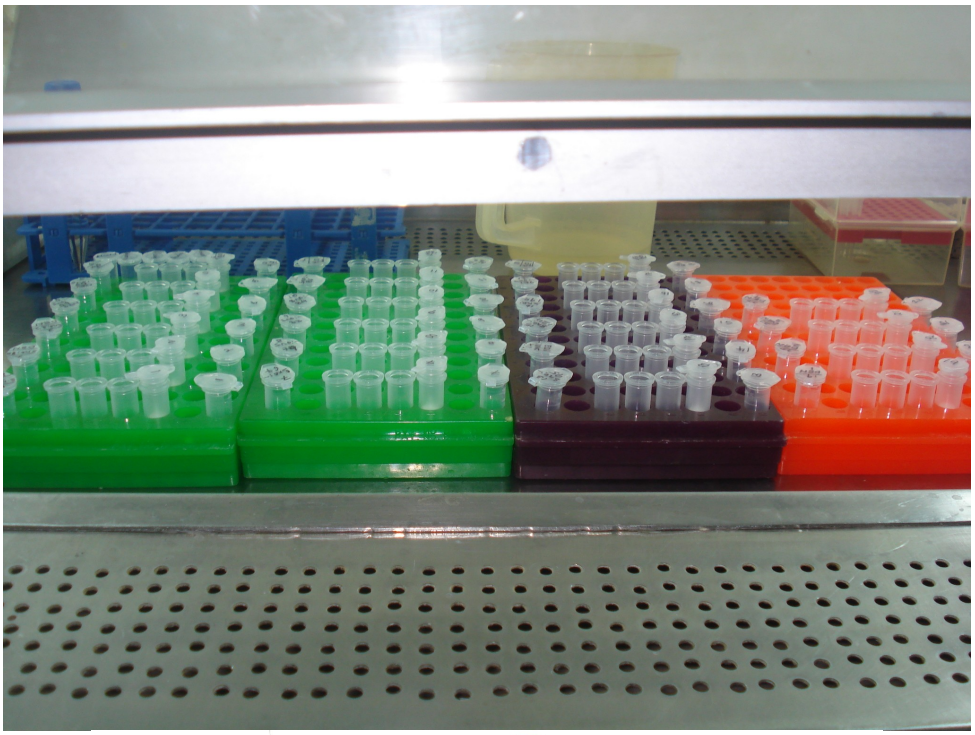
Both +ve IgM +ve IgG +ve Both -ve

elisa washer and spectrophotometer

ddengue elisa

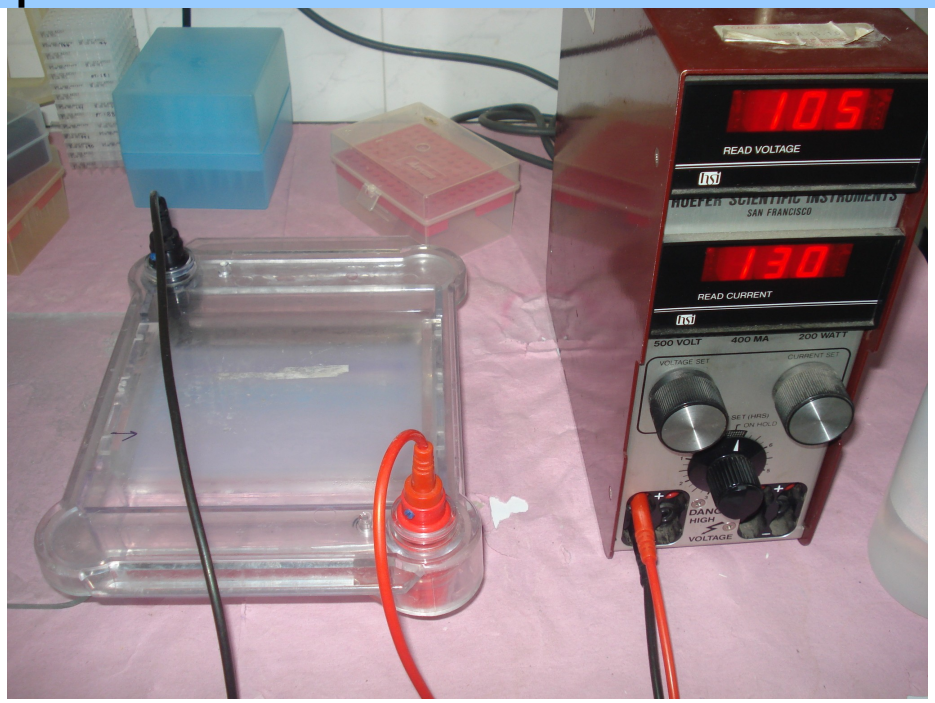


dengue rna extraction kit



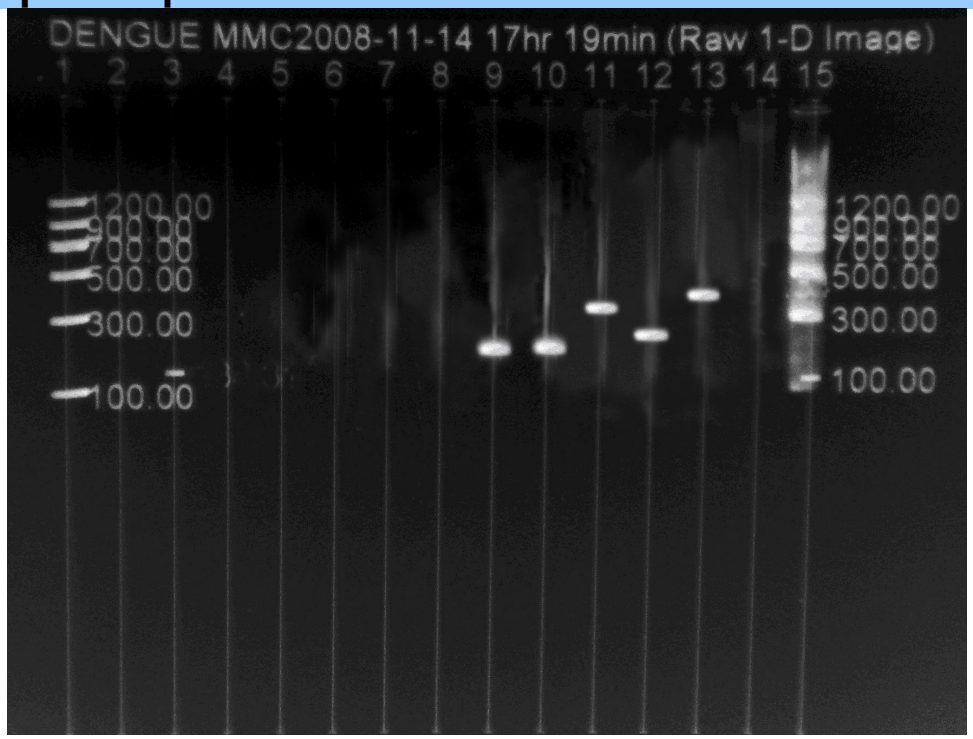
Thermal cycler

gel electrophoresis



uv transilluminator

single step RT – pcr documentation



Discussion

DISCUSSION

Dengue has been increasingly recognized as an emerging infectious disease for the last four decades. The global burden of dengue has grown dramatically in recent years.

The high presvalence of dengue cases at Chennai in the recent years, makes it necessary to evaluate the incidence of dengue and to find out the seropositivity of dengue cases.

Rapid diagnosis of dengue is crucial for proper patient care. As IgM antibody appears early during the disease course, its detection is a valuable tool for rapid diagnosis.

This study was done with 250 serum samples from patients with clinical symptoms suggestive of dengue.

The incidence of dengue fever in this study, over a period of one year (from October 2006 to October 2007) was 0.88% (Table-1), among fever cases attending the fever clinic and those admitted in our hospital.

The seropositivity of dengue cases (Table-2) in the present study among clinically suspected fever cases was 36%.In the study conducted by Khoa TD et al, in 2005 at Vietnam, the seropositivity was 53% [34].

A higher distribution of dengue cases in the present study was seen in the (Table-3) 21-40 year age group 51(46.36%), followed by 0-20 age group 31(43.05%) and above 40 age group 8(11.76%).This was similar to the study conducted by Preeti Bharaj et al in 2008, in which the common age group involved was 20-40 years(35.4%) , followed by 0-20 years

group(20.8%).[72] Ekta gupta et al, in 2006, in her work also showed that the maximum number of cases in a 3 year study period was seen in the 21-40 year age group. [11]

In this study, an increased incidence of dengue was found among male patients 51(42.85%), as compared to females 39 (29.77%) (Table-4). In the study done by Nadeem Sajjad Raja et al in 2006, they observed an incidence of 51.55% in males and 48% in females[58], and in the study by Kurukumbi et al in 2001[42], it was observed that the incidence was 61.5% in males and 38.5% in females.

The predominant symptoms with which the patients presented in the present study (Table-5) were fever(100%), myalgia/ arthralgia (71.1%), haemorrhagic manifestations (38.88%), headache (48.88%), Rash (27.77%),gastro-intestinal symptoms (22.22%),hepatomegaly (16.66%) and retro-orbital pain(13.33%).In the study conducted by Min-Shen Lee et al in 2005, a one year study involving 1551 patients in Taiwan, fever was the most common symptom (96.1) , followed by myalgia (68.5%), headache (55.4%), skin rash (53.7%) and retro-orbital pain (15.8%), which correlated well with the present study [52]. In a 16 months study by Shahid Ahamed et al in2008[83], involving 5200 fever cases, they showed that fever was the commonest symptom (100%), followed by, myalgia (67%), headache (54%) and rash (28%).

Haemorrhagic manifestations (Table-6) were seen in 35 cases (38.88%), in the present study, which included petechiae in 8 cases(22.85%), bleeding gums in 9 cases(25.71%), epistaxis in 5 cases(14.28%), hemetemesis in 5 cases (14.28%), malena in 5 cases (14.28%), ecchymosis in 1 case (2.85%), and vaginal bleeding in 2 cases (5.71%). Leukopenia was seen in 52 (57.77%) patients(Table-12), thrombocytopenia (Table-8) with

platelet count of less than 1 lakh was seen in 81 cases (90%) and more than 1 lakh in 9(10%)cases. In a study by Shahid Ahmed et al, in 2008, gum bleeding & epistaxis were seen in 40%, hemetemesis in 22%, malena in 14%, leucopenia in 73% and thrombocytopenia in 84% of cases, which closely resembles our values [83]. In the study done by Min-Sheng Lee et al, in 2005 at Taiwan, they observed that haemorrhagic manifestations were present in 73% of patients, with bleeding from skin and mucosal sites of 70.6%, leucopenia sin 55% and thrombocytopenia in 78.9% of patients[52].

The incidence of dengue is higher following rainfall. True to this, in the present study, a clear cut increase in incidence of dengue cases (Table-7) was seen between August to December when TamilNadu receives rainfall from North East monsoon. In a Laboratory based study on dengue fever surveillance conducted by John Victor et al in 2007, the data on monthwise incidence of dengue in Tamilnadu for the past nine years revealed that the number of cases increased from June to December, confirming that the active transmission period is during monsoon and post–monsoon period every year[31].In a study by Kurukumbi M et al, in 2001 done for a period of one year at AIIMS, New Delhi, and it was observed that the peak incidence of dengue infection occurred between September and November, which shows results similar to the present study. [42]

According to WHO guidelines[101] (Table-10 &11), dengue cases in the present study were categorized into dengue fever(DF) 45(50%), dengue haemorrhagic fever(DHF) 38(42.22%) and dengue shock syndrome(DSS) 7(7.7%). Preeti Bharaj et al, in 2008 in her study in New Delhi, showed 53.84% cases with dengue fever, 35.89 % with dengue haemorrhagic fever and 10.25% with dengue shock syndrome, which showed slightly similar results to the present study [72].

In the present study, an increased detection of IgM antibody (66.66%) was seen in the early febrile period (1-5 days) (Table-9), as compared to the late period (6-10 days) , when both IgM and IgG antibodies are seen at higher levels (68.96%). IgM antibodies were detected in 37.7% of the samples and IgG in 22.22% and both IgM & IgG were detected in 51.11% of patients in this study. In the study by Neeraja et al in 2006, done at Andhra Pradesh, IgG was detected in 40.28% cases, IgM in 3.8% of cases and both IgM & IgG in 55.9% of cases[60]

Elevations of hematocrit to ≥ 20% (Table-12) were seen in more than half of the subjects with dengue haemorrhagic fever. Elevated AST levels, elevated bleeding time and clotting time and hypoprotenemia were seen more in DHF patients. In the study done by Kalayanarooj et al, in 1997 at Bangkok, elevation of hematocrit and other parameters were seen in half of the subjects with DHF [33].

Platelet transfusions(Table-12) were given for 3 patients with DF, 20 patients with DHF and 7 patients with DSS, a total of 29 (32.22%) patients in the present study. In the study conducted by Malavige et al, in 2006 at Srilanka, platelet transfusions were needed for 38% of patients with severe thrombocytopenia[48.] In Makroo et al's stuty in 2007[47], platelet transfusions were given to 42.6% of patients[47.]

In the present study Rapid dengue duo cassette method, IgM ELISA, and IgG ELISA were done for 250 samples (Table-13). Rapid test was positive for 86 samples(34.4%), IgM ELISA in 80 samples (32%) and IgG ELISA in 66 samples (26.4%). The detection of dengue cases was more by rapid test method when compared to the other two methods in this study.

In the study by Satish et al., at CMC Vellore in 2003, they also found that the detection of dengue was more by Rapid tests than ELISA.[80]

Single-step nested RT-PCR was done at CMC, Vellore for 15 samples (Table-14) in the early febrile period which were positive for IgM antibody , but viral genomes could not be detected in those samples. RT-PCR was repeated with 13 other samples in the early febrile period, which were negative for IgM and IgG antibodies, collected from patients clinically suspected of having dengue fever. Dengue serotype-1 was detected in one sample with history of fever for two days. This could be due to the fact that , viremia declines soon after antibodies start appearing [10, 6]. There is also a possibility that patients reporting to General Hospital usually present at the late febrile period since this is a tertiary care hospital, the time by which the chances of detection of virus is very low. Hence, much earlier samples, very soon after fever occurs, should be collected and subjected for dengue PCR for detection of the viremia and for serotyping studies.

Dengue serotype 1 was detected in one sample among 13 samples by Single step nested RT-PCR in this study(Table-15). In the study by Kukreti et al., in 2006 at Delhi, a sudden emergence of dengue virus serotype 1 was observed in the 2006 outbreak [40].

In the present study, the proportion of primary dengue infections to secondary dengue infections was **1:2.5** (Table-17), with primary infections in 28.88% of patients and secondary dengue infections in 71.11% of cases .In Malavige et al's study conducted in 2006, they observed that 34.3% patients presented with primary dengue and 65.7% with secondary dengue which correlates with the present study[48] .Ole Wichmann et al, in 2004 at Humbolt university, Berlin, Germany documented 79.5% of secondary infections in his study, which

was very close to the present study [61].

Case fatality rate seen in the present study was 2.2%(Table-18). This was similar to the study conducted by Nazish Butt et al, in 2007, who observed a case fatality rate of 2.88% [59]. The WHO fact sheet 2002 also shows a statistics of 2.5% case fatality rate each year among dengue cases, which coincides well with the present study [10].

Summary & Conclusion

SUMMARY

- 250 clinically suspected cases of dengue were included in the study.
- Rapid dengue duo cassette method, IgM ELISA , IgG ELISA and Single-step nested RT-PCR tests were done for the suspected fever cases.
- Incidence of dengue among fever patients in this study was 0.88%.
- The common age group of patients presenting with dengue was 21-40 years.
- Preponderance of dengue was found in males (42.85%) as compared to females (29.77%).
- Fever was the most common presenting symptom (100%), followed by myalgia/arthralgia (71.1%), headache (48.88%), haemorrhagic manifestations (38.88%), rash (27.77%), gastro-intestinal symptoms (22.22%), hepatomegaly (16.66%) and retro-orbital pain (13.33%).
- The common haemorrhagic manifestations among dengue patients were gum bleeding and petechiae.
- Increased incidence of dengue was found during August to December months, during monsoon and post monsoon period.
- Thromboctyopenia was seen in all the dengue cases and most of the cases had a platelet

count of 50,000 to 1 lakh/cu.mm.

- Out of 250 samples tested, 30 patients (66.66%) were found to be positive for IgM antibodies in the early febrile period as compared to IgG antibodies (6.66%).
- During the late febrile period, both IgM and IgG antibody positivity was found to be increasing (68.96%).
- Dengue cases were categorized according to WHO criteria into dengue fever (50%), dengue haemorrhagic fever (42.22%) and dengue shock syndrome(7.7%).
- Haemorrhagic manifestations (38.88%) were seen more in dengue haemorrhagic fever cases, with thrombocytopenia of < 50,000 platelets/cu.mm .
- Rapid duo cassette method for dengue showed a higher case detection than ELISA methods.
- Dengue serotype 1 was detected in one case by Single-step nested RT-PCR in the early febrile period.
- The proportion of secondary dengue infection was more than primary dengue infection.
- Case fatality rate was found to be 2.2% in this study.

CONCLUSION

- The incidence of dengue was **0.88%** among patients attending the fever clinic and patients admitted with fever in Government General Hospital, Chennai.
- The seropositivity of dengue cases was **36%.**
- The dengue cases were classified according to WHO guidelines and were found to be **50% of Dengue fever cases, 42.22% of Dengue haemorrhagic fever cases** and **7.7% of Dengue shock syndrome** cases.
- The proportion of primary dengue infections (28.88%) to secondary infections (71.11%) was **1:2.5.**
- The serotype of dengue virus isolated in the present study belonged to **Dengue virus serotype-1**.
- Serum samples should be collected at a very early period (1-3 days) within onset of fever to determine the dengue serotype by Single-Step Nested RT- PCR.
- Serological diagnosis should be done in all clinically suspected dengue cases for early initiation of treatment and thereby to minimize the mortality.

Annexure

APPENDICES

1. IgM ELISA

Antigen preparation

Antigen is supplied in liquid form,

constituents per strip:

Antigen	2µl
Antigen diluent	500µl
Tracer(Conjugate)	502µl
Total	1004µl

Wash buffer

Constituents per strip:

Wash buffer concentrate	2.5ml
Distilled water	47.5ml
	50 ml

2. IgG ELISA

Antigen preparation

Antigen supplied in lyophilized form

Constituents per strip:

Reconstituted antigen	500µl
Mab Tracer	500µl
	1000µl

3. PCR BUFFERS

TAE BUFFER

Constituents :

Tris base	54g
Boric acid	27.5ml
EDTP(0.5M)	20ml
pH	8.0

1X PCR Buffer

Constituents:

Magnesium chloride ($MgCl_{2)}$	1.5mM
Tris-Hydrochloride (HCl)	50mM
Potassium chloride(KCl)	75mM
pH	8.3

PROFORMA

Laboratory Investigation Form for Dengue Infection

Hospital:……Reg.no……

Name of the patient:…….Age:…….Sex:……

Date of Admission:……..... Date of Onset:……..

Suspected Diagnosis:……..

Clinical Findings:

1. Fever Duration……..Days…….

2. Petechiae…….Epistaxis…….Malena.....

3. Bleeding time:……

4. Clotting time:……...

5. Platelet Count……..

Specimen No:……

Date of Collection:……

Result of Serology:……...

ABBREVIATIONS

BHK21	-Hamster kidney cells
DENV	-Dengue virus
DF	-Dengue Fever
DHF	-Dengue haemorrhagic fever
dNTP	-Deoxynucleotide Triphosphate
DSP	-Dengue Structural protein
DSS	-Dengue Shock Syndrome
ELISA	-Enzyme linked immunosorbent assay
LLCMK2	- Rhesus monkey kidney cells
Mab	-Monoclonal antibody
MAC-ELISA	-IgM antibody capture Elisa
NASBA	- Nucleic acid sequence-based analysis
PBS	- Phosphate Buffered Saline
RNA	- Ribonucleic acid
rpm	- revolutions per minute
RT-PCR	- Reverse – Transcriptase Polymerase Chain Reaction
Vero	- African green monkey kidney cells
WHO	- World Health Organisation

INSTITUTIONAL ETHICAL COMMITTEE
GOVERNMENT GENERAL HOSPITAL & MADRAS MEDICAL COLLEGE,
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Fax : 044 – 25305115

K.Dis.No.16328 P & D3/Ethies/Dean/GGH/08 Dated: **08.09.2008**

Title of the work : **A STUDY ON INCIDENCE AND SEROTYPING OF DENGUE IN A TERTIARY CARE HOSPITAL.**

Principal Investigator : **Dr.C.S. Sripriya**

Department : **Institute of Microbiology, MMC, Chennai - 3**

The request for an approval from the Institutional Ethical Committee (IEC) was considered on the IEC meeting held on 10th Sept. 2008 at 2 p.m. in GGH, Dean's Chamber, Chennai - 3

The members of the Committee, the Secretary and the Chairman are pleased to approve the proposed work mentioned above, submitted by the principal investigator.

The principal investigator and their term are directed to adhere the guidelines given below:

1. You should get detailed informed consent from the patients/participants and maintain confidentiality.
2. You should carry out the work without detrimental to regular activities as well as without extra expenditure to the Institution or Government.
3. You should inform the IEC in case of any change of study procedure, site and investigation or guide.
4. You should not deviate form the area of the work for which I applied for ethical clearance.
5. You should inform the IEC immediately in case of any adverse events or serious adverse reactions.
6. You should abide to the rules and regulations of the institution(s)
7. You should complete the work within the specific period and if any extension of time is required, you should apply for permission again and do the work.
8. You should submit the summary of the work to the ethical committee on completion of the work.
9. You should not claim funds from the Institution while doing the work or on completion.
10. You should understand that the members of IEC have the right to monitor the work with prior intimation.

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