CORRELATION OF NS1 ANTIGEN TITRES WITH THE SEVERITY OF DENGUE FEVER IN CHILDREN

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In partial fulfillment of the requirements for the degree of

Doctor of Medicine in Paediatrics

Under the guidance of

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Tamil Nadu Dr. M.G.R Medical University, Chennai

MAY 2018

CERTIFICATE BY THE HOD AND DEAN OF THE INSTITUTION

This is to certify that the thesis entitled **"CORRELATION OF NS1 ANTIGEN TITRES WITH THE SEVERITY OF DENGUE FEVER IN CHILDREN"** is the bonafide original research work of **Dr.LAVANYA .M**, done under the guidance of **Dr. A. JAYAVARDHANA**, Professor of Paediatrics PSG IMS&R, Coimbatore in fulfilment of the regulations laid down by The Tamilnadu Dr.M.G.R Medical University for the award of MD degree in Paediatrics.

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DECLARATION

I, hereby declare that this dissertation entitled **"CORRELATION OF NS1 ANTIGEN TITRES WITH THE SEVERITY OF DENGUE FEVER IN CHILDREN"** was prepared by me under the guidance and supervision of **Dr. A. JAYAVARDHANA,** Professor, Department of Paediatrics, PSG IMS&R, Coimbatore.

This dissertation is submitted to The Tamilnadu Dr.M.G.R Medical University, Chennai in fulfilment of the university regulations for the award of MD degree in Paediatrics. This dissertation has not been submitted elsewhere for the award of any other Degree or Diploma.

Dr. LAVANYA .M

CERTIFICATE-II

This is to certify that this dissertation work titled **"CORRELATION OF NS1 ANTIGEN TITRES WITH THE SEVERITY OF DENGUE FEVER IN CHILDREN"** of the candidate **Dr. LAVANYA .M,** with registration Number **201517501** for the award of DOCTOR OF MEDICINE in the branch of PAEDIATRICS. I personally verified the urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows **2%** of plagiarism in the dissertation.

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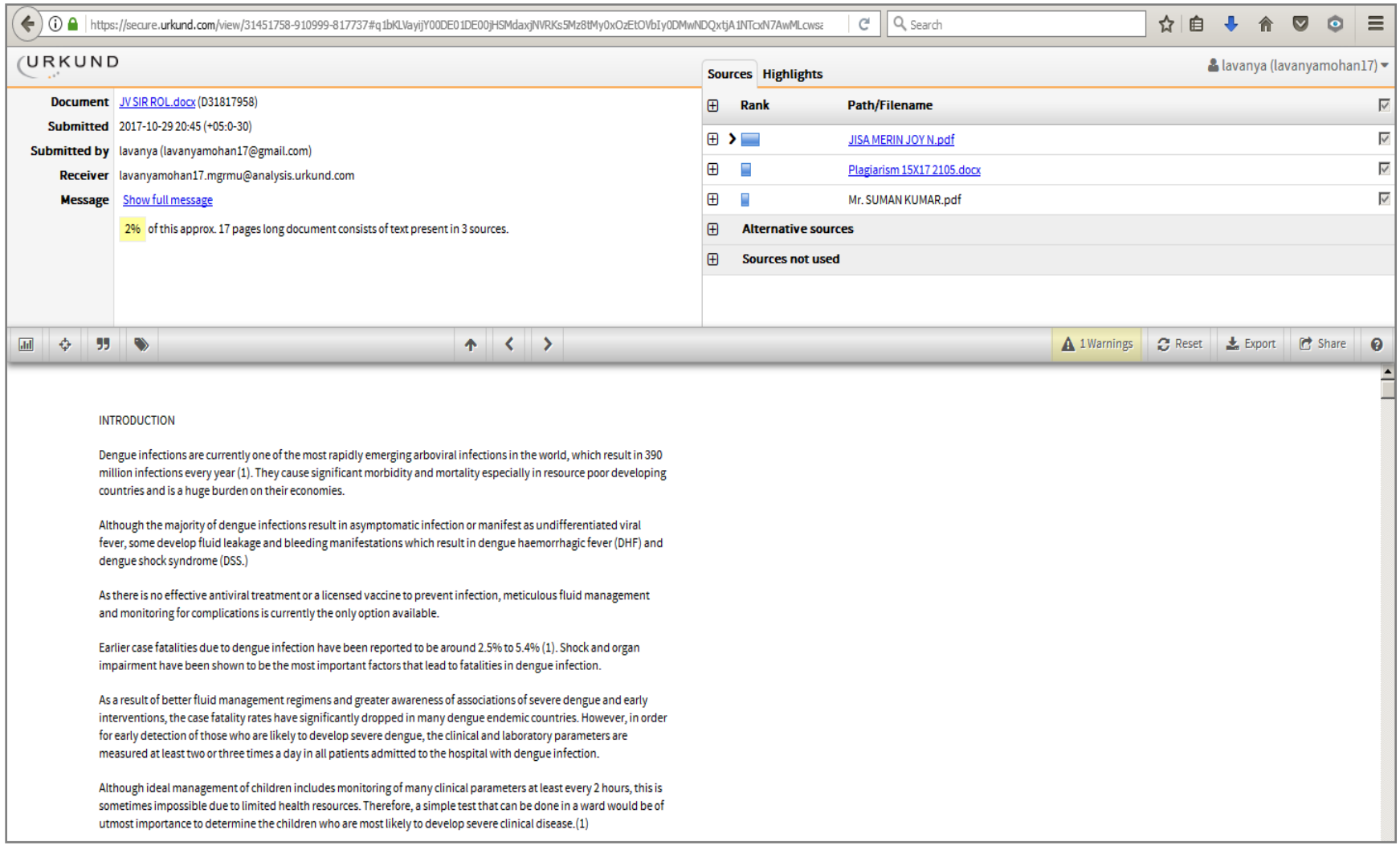
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INTRODUCTION
Dengue infections are currently one of the most rapidly emerging arboviral infections in the world, which result in 390 million infections every year (1). They cause significant morbidity and mortality especially in resource poor developing countries and is a huge burden on their economies.
Although the majority of dengue infections result in asymptomatic infection or manifest as undifferentiated viral fever, some develop fluid leakage and bleeding manifestations which result in dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS.)
As there is no effective antiviral treatment or a licensed vaccine to prevent infection, meticulous fluid management and monitoring for complications is currently the only option available.
Earlier case fatalities due to dengue infection have been reported to be around 2.5% to 5.4% (1). Shock and organ impairment have been shown to be the most important factors that lead to fatalities in dengue infection.
As a result of better fluid management regimens and greater awareness of associations of severe dengue and early interventions, the case fatality rates have significantly dropped in many dengue endemic countries. However, in order for early detection of those who are likely to develop severe dengue, the clinical and laboratory parameters are measured at least two or three times a day in all patients admitted to the hospital with dengue infection.
Although ideal management of children includes monitoring of many clinical parameters at least every 2 hours, this is sometimes impossible due to limited health resources. Therefore, a simple test that can be done in a ward would be of utmost importance to determine the children who are most likely to develop severe clinical disease.(1)

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CONTENTS

INTRODUCTION

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Although the majority of dengue infections result in asymptomatic infection or manifest as undifferentiated viral fever, some develop fluid leakage and bleeding manifestations which result in dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS.)

As there is no effective antiviral treatment or a licensed vaccine to prevent infection, meticulous fluid management and monitoring for complications is currently the only option available.

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early detection of those who are likely to develop severe dengue, the clinical and laboratory parameters are measured at least two or three times a day in all patients admitted to the hospital with dengue infection.

Although ideal management of children includes monitoring of many clinical parameters at least every 2 hours, this is sometimes impossible due to limited health resources. Therefore, a simple test that can be done in a ward would be of utmost importance to determine the children who are most likely to develop severe clinical disease.

Early diagnosis and prompt treatment can help in reducing significant mortality and our study has attempted to identify a predictor of severe dengue.

AIM AND OBJECTVES

AIM

To assess the role of NS1 antigen Assay in the management of dengue fever in children

OBJECTIVE

To assess the utility of NS1 antigen assay in predicting severity of dengue fever in children by estimating titres.

MATERIALS AND METHODOLOGY

STUDY DESIGN: Hospital based prospective observational study

PLACE OF STUDY: PSG Hospitals, PSGIMS&R , Coimbatore

TIME PERIOD: January 2016 to May 2017

SAMPLE SIZE: 250 samples were required based on the formula 4p9/d2 considering the prevalence of dengue fever

INCLUSION CRITERIA:

- Age: less than 18 years
- Study was carried out in children with clinical suspicion of dengue fever with features like
- Fever > 3 days
- Myalgia
- Rash
- Arthralgia

EXCLUSION CRITERIA

- Children with dengue fever and co infections were excluded .

METHODOLOGY

Ethical committee approval was obtained and Prospective collection of data was done in children who fulfilled the inclusion criteria in the Pediatrics department at tertiary care centre, PSGIMSR, Coimbatore where systematic computer coding for registry is used. NS1 Antigen ELISA was done in all children between day 1 to 5 illness who fulfilled the inclusion criteria and 2ml of blood was drawn and collected in EDTA container. NS1 Antigen ELISA was tested using (J-Mithra kit)India at the Serology lab in our hospital. Qualitative and semi Quantitative analysis was done.

Titre of >11 was considered strongly positive, 9-11 as equivocal and titre value of 9 was mild positive. Dengue Serology for IgG antibodies for secondary infection and IgM antibodies for primary infection were performed on the same children using ELISA Assay. All other relevant and additional investigations were done as per the course of illness.

Data was entered in structured proforma and case definition, diagnosis and management used for dengue fever were categorized into Mild, Moderate and Severe dengue as per revised NVBDCP guidelines. All other relevant clinical data were collected.

Table 1: DENGUE CASE CLASSIFICATION:

Mild dengue – Fever and any two of the following-	**Moderate dengue**	**Severe Dengue**
-Nausea, vomiting -Rash -Aches and pains -Leucopenia -Positive tourniquet Test	- Abdominalpain/Tenderness -Persistent vomiting -Ascites/ pleural effusion -Mucosal bleeding -Lethargy, restlessness -Liver Enlargement >2 cm Laboratory : Increase in HCT concurrent with rapid decrease in Platelet count	Severe plasma leakage leading to- -Shock -Fluid accumulation with respiratory distress -Severe Bleeding Severe organ Involvement -Liver AST or ALT >/= 1000 CNS:Impaired Consciousness

Figure 1: Shows the J Mithra kit used for estimating NS1 Antigen ELISA Titres

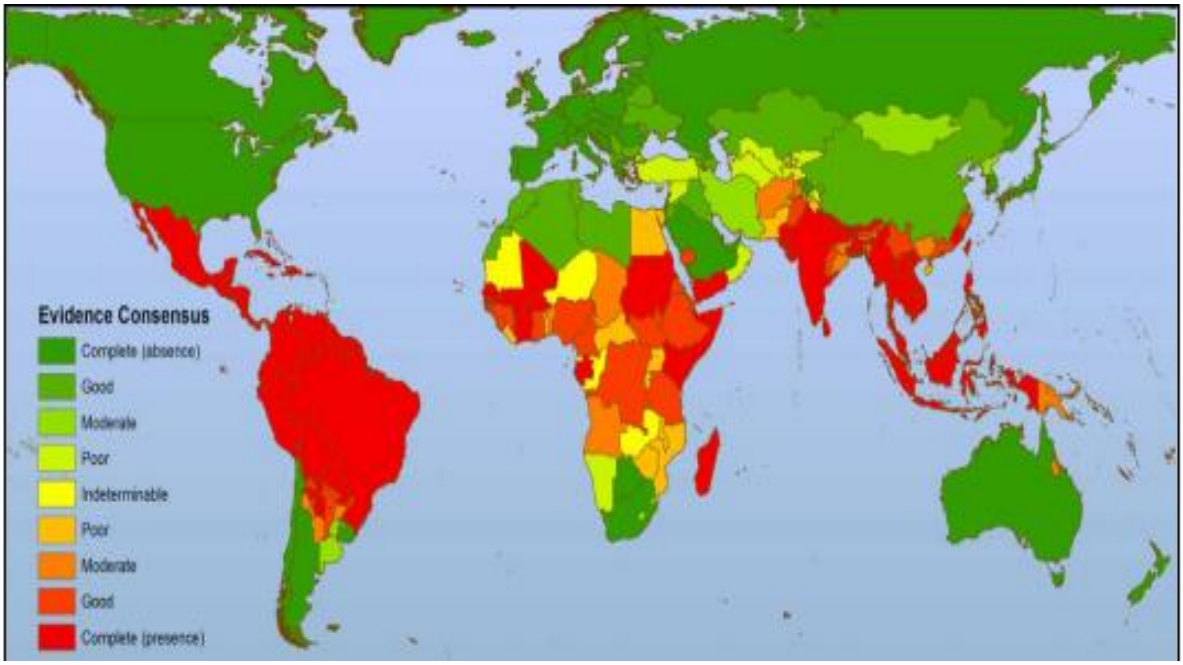
Statistical tools:

The data collected from the patients were tabulated using Microsoft Excel. The SPSS 23 statistical software was used for data analysis. After collecting all the data, all the variables were summarized by descriptive statistics. Categorical variables were expressed as frequencies and percentages, and Descriptive analysis was done using chi square test and p value of <0.05 was considered statistically significant.

REVIEW OF LITERATURE

Dengue infection is one of the widely spreading disease that is present all over the world. It is one of the mosquito borne diseases. The causative organism is the dengue virus which has 4 different variants. Increased amount of rainfall along with abundance of the vector which causes the spread of the disease results in epidemics. There are many endemic regions all over the globe where the disease is shielded. Wide distribution of the disease is seen in the Asia Pacific regions which exactly coincides with the number of developing countries of the world. The number of cases each year is on the rise. The first individual of dengue infection was reported in 1789. The first person to report the case was Benjamin who gave a special name to the disease – "the breakbone fever" .The burden of the disease has increased markedly in the world that in the year 2012 the world health organisation classified the disease as "the most important viral disease that is transmitted by the mosquitoes"

Figure 2: National and subnational evidence consensus on complete absence (Green) through to complete presence (Red) of Dengue



Dengue viruses originates from animal reservoir. Two distinct DENV transmission cycles are recognised (Figure 3)

- endemic/ epidemic cycle and
- sylvatic /zoonotic cycle.

Endemic and epidemic cycles involve the human host and viruses are transmitted by *A. aegypti, A. Albopictus* and other mosquitoes as secondary vectors The sylvatic transmission cycle involves monkeys and several different Aedes mosquitoes identified in Asia and West Africa.

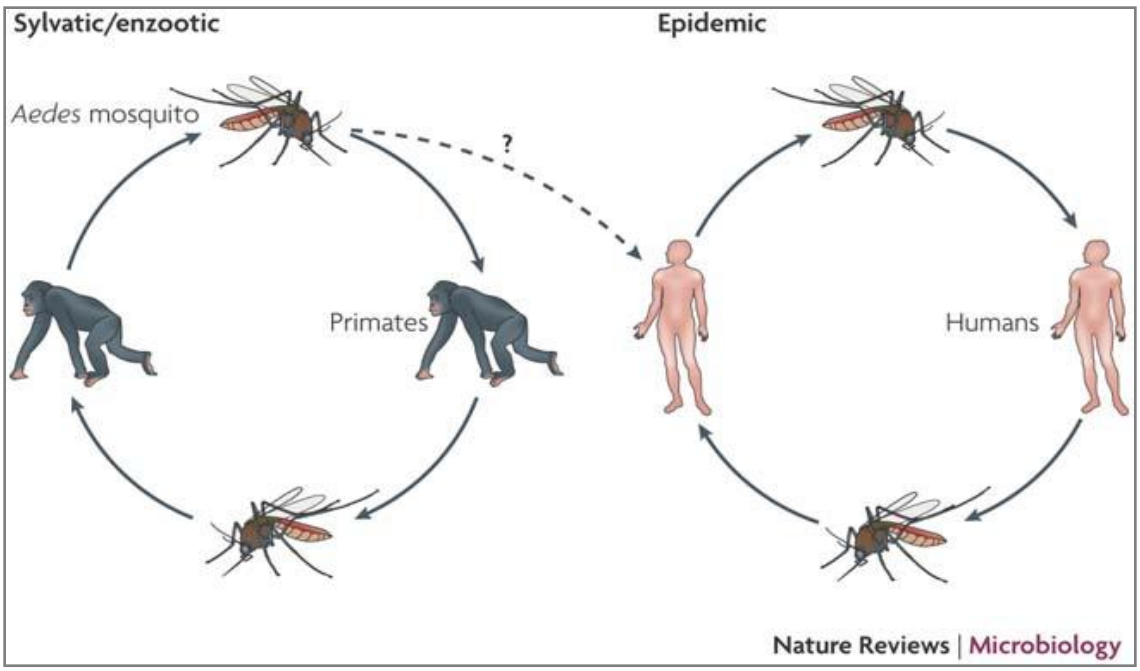


Figure 3: The Two Denv Transmission Cycles

To clearly understand the real burden of the disease the geographical location of the disease should be studied in detail. The dengue infection has more prevalence in the tropical region and many of the areas that are categorized as high risk areas come under Asia.

Temperature of a particular region plays a major role in the survival of the vector which is the major mode of transmission. The socioeconomic status also aids with the spread of infection

India was challenged with the first epidemic(not proven virologically but only by clinical features) in Chennai and later in Calcutta which was proven virologically in the year of 1963. Delhi the most predominant type of dengue virus

was of type 2 and 3 but recent trends show type 1 as the most prevalent type in the city of Delhi.

Dengue is mostly spread by the bite of day time mosquitos in contrast to other disease vectors. Stagnation of clean water is the suitable place for breeding of these vectors.

Individuals once exposed to a mosquito bite the virus enters the body through systemic circulation and lodges in the lymphatics and from there gets disseminated to various organ systems of the body. It affects many systems of the body but most significant among them are reticuloendothelial system, endothelial cell lining the various blood vessels in the body.

The exact mechanism by which there is varied clinical spectrum of the same disease is not understood clearly, but through various studies though not fully much more information regarding the pathogenic aspect of the disease has been tried to figure out recently.

Hypothesis had been stated that the imbalance between the inflammatory cascade which is triggered by the infection and anti inflammatory pathway is responsible for the disease to manifest in various severity levels. Infection leads to increased production of inflammatory mediators and increased destruction of endothelial cells leading to the destruction of platelets all of leading to plasma

leakage which holds responsible for most of the clinical manifestations of the disease.

The clinical spectrum which ranges from mild disease to dreadly severe form of the disease can be classified broadly into

NON SEVERE FORM

- Asymptomatic infection
- Classical dengue fever

SEVERE FORM

- Dengue hemorrhagic fever
- Dengue shock syndrome

CLINICAL FEATURES:

Common symptoms

- Fever
- Headache ,arthralgia, malaise, fatigue
- Generalized maculopapular rash – sometimes associated with itching
- Facial flushing
- Conjunctival congestion
- Gum bleeding

Rare features:

Neurological Manifestations (seen in severe form of the disease):

- Neck rigidity
- Pyramidal signs
- Myoclonus
- Manic psychosis
- Hepatic failure leading to encephalopathy

Gastrointestinal manifestations:

- Hepatitis
- Rarely Fulminant hepatic failure
- Acute pancreatitis
- Diarrhoea
- Gall bladder wall edema and thickening

Cardiovascular manifestation:

- Av blocks
- Atrial fibrillation

DIAGNOSIS

Dengue fever:

Defined as fever whose duration is between two days and one week with prodromal symptoms like head ache, malaise,rash etc

Dengue haemorrhagic fever:

All of the above criteria with significant haemorhagic manifestations, significant drop in platelet count less than 1 lakhs and with evidence suggestive of severe plasma leakage

Dengue shock syndrome:

Includes all the above listed criteria with features of circulatory failure enough to cause compensated shock.

Laboratory diagnosis:

- Ns1 antigen detection test
- IgM antibodies that are specific to dengue
- RT-Polymerase chain reaction
- Culture of the virus

Management:

There is no specific antiviral therapy available till now for the disease. Management mainly involves fluid replacement therapy and management of the complications of the illness.

Mild form of the disease is treated with antipyretics of which aspirin and other NSAIDS are not preferred and hydration theraphy should be the main stay of treatment.

Periodic monitoring of haematocrit, platelet counts should be done Intramuscular injections are avoided because of the thrombocytopenia which can cause significant haematoma. Significant thrombocytopenia of less than one lakh should be considered for hospitalization.

In severe dengue children IV fluids is the main modality of treatment followed by symptomatic management. If there is significant bleeding whole blood and Fresh frozen plasma should be the preferred treatment if clinical suspicion of Disseminated intravascular coagulation is suspected. Elective intubation should be planned if presentation is of acute respiratory distress syndrome is clinically suspected.

Dengue is one of the significant vector borne disease,which is spread from person to person through group of mosquitoes –Aedes.

The DENV is a vector-borne virus transmitted to humans primarily by bites from two mosquito species,AedesAegyptior and Aedes albopictus. DENV is a single positive-stranded RNA virus belonging to Flavivirus genus of the Flaviviridae family.

4 major serotypes (DENV1–4) that are antigenically distinct from each other. Each DENV serotype is distinct suggesting that each serotype could be considered a separate virus.Dengue is more common in the developing nations. Currently there are no particular empirical/prophylactic medications or any other vaccinaton available to treat dengue.

Many similar infection like those of chikungunya and recent epidemic causing zika virus also make the diagnosis difficult as it has similar clinical manifestations. The best method of preventing the disease occurrence is the vector control. [(2)]

Dengue outbreak is difficult to predict and limited models established for predicting the onset of the disease particularly in developing nations,tropical countries.[(2)]

Age of people getting affected also varies considerably in various countries. In Asian countries pediatric age group tends to get severe disease whereas American continents adult age group is the one which is mainly affected.[2]

The severity of Dengue infection can differ in every individual sometimes causing mild less symptomatic disease known as dengue fever which mimics other flu like infections to severe form of infection called dengue haemorrhagic fever and dengue shock syndrome. Both presentations are the different spectrum of the same diseases.

Dengue can be spread through transfusion of the blood or any of its components /organ transplantation which is already contaminated with the virus [2]

HISTORY OF DENGUE

Dengue has been known by several names throughout the world, the term 'dengue' has been universally adapted. The origin of the term "dengue"comes from the Swahili word for the disease "ki-dingapepo". The earliest description of an illness called "dengue" can be found in Spanish written records from 1800.[3,4]

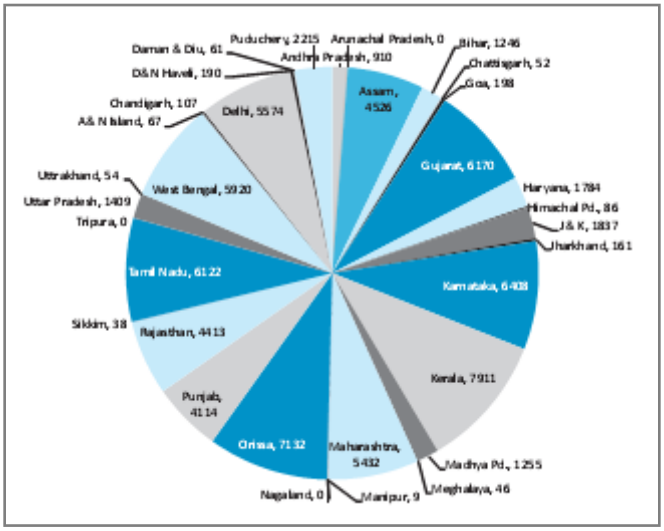
The term "denga", or "dyenga," was coined during the disease outbreaks in East Africa and West Indies during the early 19th century. After the 1828 outbreak in Cuba that the Spanish word "dengue" came into general use, which is accepted universally now.

The earliest historical evidence of dengue like illness were found in China in 992 A.D. They described the disease as ‘water poison’ and found to be associated with flying insects. The first epidemic of a disease resembling symptoms of dengue was reported in Philadelphia, in 1780.

In the year 1906, Thomas Lane Bancroft discovered that the mosquito vector *Aedesaegypti* transmitted dengue. In 1907, Ashburn and Craig proved that dengue was caused by a virus.Japanese scientists were first to discover the DENV-1 in 1943. Subsequently, in 1944, DENV-2 was discovered by Albert Sabin.The first reported cases of the severe form of dengue (Dengue Haemorrhagic Fever) were seen in Thailand and Philippines during the 1950s.

The history of dengue outbreaks in India has been recently studied by the NVBDCP

Figure-4 shows the distribution of dengue cases among the states of India in 2013

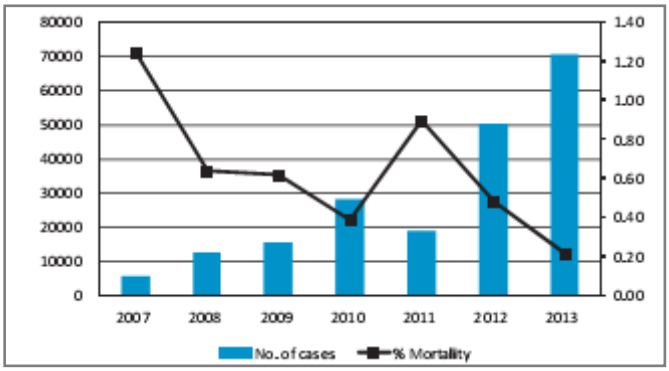


Mortality in dengue has been reduced significantly even though number of cases being reported is increasing considerably every year.

The overall mortality rate of 1.2% in 2007 dropped to 0.25% in 2013, compared with the rest of South-East Asia, the number of dengue shock syndrome (DSS) cases in India remains low.

A pilot age-stratified, cross sectional dengue prevalence study was carried out at KEM Hospital Pune which revealed significantly higher seropositivity of 58.5% for DENV was found in the urbanized village, compared with 41.2% for the rural village.(5)

Figure 5 Total dengue cases reported to NVBDCP (left axis) and percentage mortality (right axis) in India, 2007–2013



Mortality in paediatric age group was studied by Cherian et al (6)which showed a case-fatality rate of 26.3% in a study that included 19 children older than 1 year in 2012 in the North Arcot district and the adjoining areas of Tamil Nadu and Andhra Pradesh.

Another study, in Mumbai in 2013[7,8] reported three deaths in 38 DHF/DSS cases in the paediatric intensive-care unit with a mean age of 4.9 years.

DENGUE IN TAMILNADU

During the recent few years the incidence of dengue hemorrhagic fever (DHF) has increased markedly in South India. The life span of the vector is strongly influenced by temperature and humidity and its survival is best between relative humidity of 60–°C and 30°. The breeding of A. aegypti fluctuates with

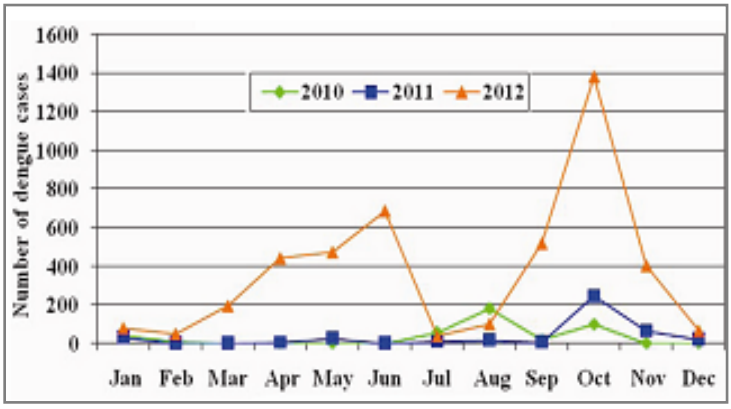
unusual rainfall pattern and water storage, as it breeds well in the open containers in and around houses.

In 2010–2012[7,8] outbreaks of dengue/ chikungunya-like illnesses with severe clinical manifestations were reported from several districts of Tamil Nadu, such as Tirunelveli, Virudunagar, Theni, Madurai, Thiruvallur, Vellore and Dharmapuri.

A study was conducted during three consecutive years(2010-2012) monthly data on rainfall, power supply and reported cases of dengue in Tamil Nadu and Puducherry. The number of dengue cases was collected from the weekly disease alert reports of the Integrated Disease Surveillance Project (IDSP).

Dengue cases showed a specific pattern of occurrence. Reports showed a peak in October followed by gradual decline reaching lowest in February

Figure 6 shows the monthly dengue cases in Tamilnadu during 2010 to 2012



Dengue outbreaks coincided mainly with the northeast monsoon, followed by the southwest monsoon with moderate rainfall. With respect to the districts, maximum cases were reported from Viluppuram district (226) in 2010, Puducherry (152) in 2011 and Puducherry (1600) followed by Tirunelveli (1365) in 2012. Deaths were high (40) in 2012, especially from the Tirunelveli district

EPIDEMIOLOGY

History of symptoms similar with dengue can be traced back to the China Dynasty of 265 Dengue is currently regarded globally as the most important mosquito-borne viral disease. A history of dengue epidemics as early as the 420 AD.[9]

The virus and its vectors have now become widely distributed throughout tropical and subtropical regions of the world, particularly over the last half-century. Significant geographic distribution has been associated with rapid rise in incident cases, epidemics, and hyperendemicity, leading to the different and more severe forms of dengue.

Transmission of dengue is now present in WHO region of the world and more than 125 countries are known to be dengue endemic.[9,10]

The true impact of dengue globally is tough to determine because of inadequate disease surveillance, misdiagnosis, and less number of cases getting

reported. Currently available data grossly underestimates the social, economic, and disease burden. Recent Estimatation of the global incidence of dengue is closer to almost 400 million[9,10]

The global wide distribution of dengue is expected to increase due to factors such climate change, globalization, travel, trade, socioeconomics, settlement and viral evolution.

Surveillance and improved reporting of dengue cases is also essential to gauge the true global situation as indicated in the objectives of the WHO Global Strategy for Dengue Prevention and Control, 2012–2020.[10]

Three dengue serotypes out of four (DENV 1–3)have been found in Middle Eastern countries including Saudi Arabia and Yemen. DENV-1 strain isolated in Saudi Arabia exhibited a high genetic similarity with DENV-1 strain isolated from Asian population, suggesting a widespread of the Asian genotype, probably through Asian pilgrims [11,12].Recent publications identified a new serotype (DENV-5).[11]

Mosquitoes transmit the virus by feeding on blood of infected persons. At first, the virus infects which replicates in the mid-gut epithelium of the mosquito and then spreads to other organs until it reaches the salivary glands after 10–14 days where it can be inoculated to another person during subsequent blood meal.

Vertical transmission of DENV in mosquitoes from mosquito to larvae has been reported by a number of research groups. In India, Angel & Joshi(2008)[11,12] reported the detection of dengue virus by in-direct fluorescence antibody test (IFAT) in laboratory grown mosquitoes originating from larvae collected from urban and rural areas

A similar study was conducted in Brazil by Martins et al. (2012)[13] confirmed the isolation of DENV-type 3 in Ae. Albopictus larvae and DENV-type 2 in Ae. Aegypti larvae [13]

Mother-to-infant transmission of dengue virus via cord blood or breast milk is not clearly studied.[14]

PATHOGENESIS OF DENGUE

Pathogenesis of the disease is not clear. Various mechanism has been proposed.Large scale research suggests that the pathogenesis of dengue haemorrhagic fever and dengue shock syndrome involves both the immune response of the host and also the virulence of the virus infecting the host.[15]

In vitro and autopsy suggested that 3 important organs are involved in pathogenesis of severe disease. Endothelialcells which form the inner liningof the blood vessels,the liver and the immune system.

Understanding the various changes occurring in the above three systems during the course of illness the overall pathogenesis is known clearly.

Once a host is infected with the virus through the bite of the infected mosquite the dengue virus gets into the blood and systemic circulation .

Langerhan cells and keratinocytes are infected initially from which it is transmitted to cells like monocyte and macrophages

This occurs once the infected langerhan cells enter the lymph nodes in which monocytes and macrophages are concentrated. Through this mechanism the infection is spread extensively and affecting various cells including several organ systems like those in spleen ,bone marrow and lymphatic system.

During reinfection with other type of dengue virus the IgG formed during the previous infection causes a complex with the current infection causing virus which is engulfed by the mononuclear cells.

Mononuclear cells get destroyed by apoptic pathways, which releases excessive inflammatory mediators.

Load of viraemia determines the extent of inflammatory response of the body.[15]

Autopsy studies have demonstrated the presence of dengue virus at various organ systems like skin,liver,spleen,kidney,brain etc.[16]

Animal studies suggests that liver is the most commonly involved organ which also is proven with the occurrence of dengue hepatitis in humans((16)

As the endothelial cell layer gets involved the microvascular bed which is near the dermal papillae is affected mainly.

Studies have also suggested the significant involvement of endothelium in the pulmonary blood vessels. Functional damage is more common than anatomical damage.

Exact mechanism by which endothelial layer involvement varies in different organ systems is not clear.

The involvement of endothelial layer and increased permeability is the main pathology involved in dengue shock.

Due to the increased vascular leakage occurring secondary to the increased permeability which is more marked in the abdominal and pulmonary vessels there is fluid collection in pleural and peritoneal cavities.

One of the important non-structural protein 1 (NS1) of the dengue virus has more affinity to the endothelial layer lining the lungs and liver and it is important in the diagnosis of the disease.[16,17]

Hypothesis says that certain strains of the virus cause more severe disease due to the virulence factor of the virus which is different for each of the 4 subtypes of the viruses

After various studies it has been suggested that high incidence of the severe form of the disease i.e dengue shock syndrome/dengue haemorrhagic fever occur more commonly occur in denv 1.[18] followed by dengue virus type 2 and dengue virus type 3.

During reinfection the disease severity is linearly associated with the time interval between the primary infection and the reinfection.And if an individual during secondary infection has acquired a different virus type from the primary infection then the risk of the individual getting severe infection is much higher.

Virulence of the virus varies with different geographical location.

Severe disease in primary infection is not that common compared to the secondary infection.

Complement pathway of the body also plays a role in the pathogenesis of the disease which causes increased levels of components like C3a ,C5a levels in individuals with DSS/DHF.[19]

Non structural protein of the dengue virus triggers formation of antibodies which binds which causes activation of the complement system and various other pro inflammatory mediators.

The antibody of IgG class is also component which causes excessive inflammatory reaction in the secondary dengue.

NS1 Proteins show some increased response of cross reaction to the endothelial lining of blood vessels and platelets which causes increased production of nitric oxide in these cells and accelerated apoptosis of these cells and also increased plasma leakage via these cells [20,21]

Components like IL6,IL8 are also enhanced due to the interaction of the antibodies with various cells of the body.

Severity of the disease can vary for each person due to the various difference between each host. The difference can be at a genetic level and also due to the difference in immunity.

Certain HLA class individuals are more prone to develop severe form of the disease like dengue shock syndrome and dengue haemmorhagic fever.Certain changes in genetic levels causes difference in formation of inflammatory mediators like polymorphism of TNF α,TGFβ leads to low threshold for formation of dengue shock syndrome/dengue haemorhagic fever.

Polymorphism in transporter protein associated with human platelet antigen also causes individuals to be more prone to develop dengue haemorrhagic fever [21]

Some of these inflammatory mediators are also being evaluated for their correlation with dengue severity and duration

Chaiyaratana et al [22] for evaluating serum ferritin levels as a predictor of severe dengue.

This study was done among 100 children in Thailand

Ferritin level value of 1200 to calculate both the sensitivity and specificity and suggested that children who had ferritin levels above this cutoff value had more chance of developing the severe form of the disease i.e DHF.

Study suggested that Ferrtin is a acute phase reactant can be used to predict the severity of the disease

Lowel et al [23] perfomed a study comprising of 54 children concluded the correlation of severity in relation to interferon gamma levels and interleukin 10 and interleukin 2 levels.

Third space fluid collection and the severity of the thrombocytopenia were also correlated

Daisy vanitha et al [24] has suggested that the severity of the disease was directly proportionate to the urinary histamine levels due to the significant rise in the mast cell activity.

Hemagglutination (HI)test is recommended by World health organization but it has certain disadvantages because of the tough methodology and it requires drawing of blood sample twice at a particular time interval. Cross reactivity with other viruses has been proved because of HI. Study done by Severine Matheus et al [26,27] formulated ELISA which was based on the theory that changes in Immunoglobulin G during the acute phase of the disease was formulated.

Desouza et al [28,29] suggested a newer ELISA screening for IgG which distinguishes between secondary and primary disease can be done by screening for avid IgG.

Study done by Matheus et al [30] showed that affinity for IgG was greater in secondary dengue compared to the primary infection and the sensitivity/specificity

derived from the study showed that it was an effective screening tool to distinguish between the two forms(primary/secondary).The efficiency of the test was proved more effficient during the convaselcent phase compared to the early febrile phase. Advantage of this test showed that it can be performed only once during the later phases whereas the former showed testing twice at two different intervals.

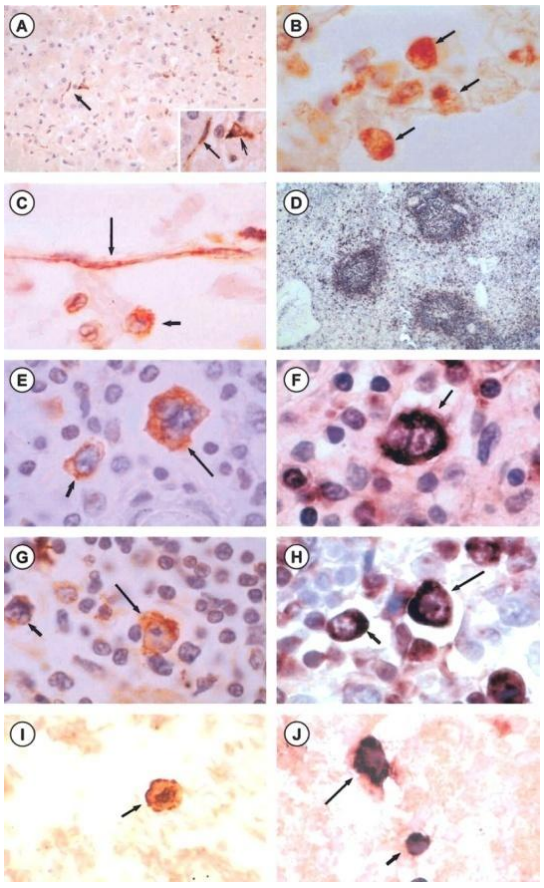
Severe dengue infection is characterized by excessive activation of the immune mediators. Lipopolysaccharide was found to be increased markedly in people with dengue infection and studies suggested that the serum levels of LPS had a good correlation with the severity of the disease.

Cornelia et al [(31)] suggested that the microbial translocation was associated with immune system activation which happens in severe dengue.This study was conducted in Brazil where levels of LPS, IgM,IgG and cd14 and many other cytokines were measured.Other markers for microbial translocation like RANTES, Interleukin 6 etc were also measured and it revealed that severity of the disease was statistically correlating with the levels of the above markers.

One study was conducted by kala jesi et al [(32)] in which the practical estimation of detection of viral RNA in tissues of the infected individuals was demonstrated. They showed the presence of the viral RNA in the macrophages present in spleen and also the monocytes of the peripheral circulation.

Methodology used to detect the viral RNA was through Immuno histochemistry and in situ hybridization. The following figure gives us the tissues in which viral RNA was detected

Viral antigens and RNA detection in various organ systems are shown in the following pictures **(Fig 7)**



A, Dengue viral antigens detected in the liver in Kupffer and endothelial cells *(arrow)*, but not in hepatocytes. *Inset*, A Kuppfer cell *(short arrow)* and an endothelial cell *(long arrow)*. Shown are dengue viral antigens in the lung in alveolar macrophages *(B, arrows)*, vascular lumen monocyte *(C, short arrow)*, and vascular endothelium *(C, long arrow)*. *D*, Dengue viral RNA localized in macrophages and reactive lymphoid cells in the red pulp and white pulp of the spleen. Shown are dengue viral antigens and RNA in the binucleated cells (*E, long arrow*, and *F, arrow*, respectively) in the red pulp, and viral antigens in a macrophage (*E, short arrow*, and *G, short arrow*). G, Viral antigens localized to an immunoblast-like cell *(arrow)*. *H*, Dengue viral RNA in a centroblast-like cell *(short arrow)* and immunoblast-like cell *(long arrow)* in the germinal centers. *I*, Dengue viral antigens in a peripheral blood monocyte *(arrow)*. *J*, Viral RNA in a peripheral monocyte *(long arrow)* and a lymphocyte *(short arrow)*. *A–C*, *E*, *G*, and *I*, Immunohistochemistry, hematoxylin counterstain; *D*, *F*, *H*, and *J*, In situ hybridization, hematoxylin counterstain. Magnification, ×100 (*A* and *D*) and ×500 (*B*, *C*, and *E–J*).

One of the fatal complication of severe dengue is the bleeding manifestation. Wills et al [33] conducted a study in dengue infected patients of pediatric age group in Vietnam Study was conducted in one sixty seven subjects who had the severe dengue.

After continuous and daily monitoring it was found out that Prothrombin time and the commonly used activated partial thromboplastin time had no significant abnormalities and had a mild prolongation of these parameters and level of fibrinogen measured was significantly low.

Protein C and protein S, the naturally occurring anticoagulants were significantly reduced.

Similar findings were also found in another natural anticoagulant i.e antithrombin. Plasminogen activator inhibitor showed marked increase in circulating levels and had significant correlation with the clinical severity of the disease

Study done by Eric et al [34] included fifty subjects and the total subjects were divided into two based on the final outcome.All the subjects were children who had DHF and there various coagulation parameters were monitored and compared to each other.A total number of 13 subjects died during the study period.

Both the coagulation and anticoagulation cascade showed increased activity in the acute phase of the disease which showed altered thrombin and antithrombins level. Among the two groups the children who survived showed an established compensated acceleration of anticoagulant cascade by means of increased natural anticoagulants like protein C and S and other factors that activate the fibrinolytic pathway activation. In the mortality group the this compensation did not occur efficiently and these children had a final effect which had more procoagulant nature resulting in more severe multiorgan system involvement.

The study group had increased levels of inhibitors of plasminogen. Results of the study suggested that procoagulant markers and the ratio of anti to procoagulant markers can be used as a marker which can guide treatment and grade severity.

Thrombocytopenia is the hall mark and very common laboratory finding in the dengue fever even in mild dengue.

Mechanism which has been proposed by various studies is that there is increase in the adhesion of platelets to the wall of the vessels due to certain changes in the endothelial layer.

In a study conducted by Krishnamurthi et al [35,36] the above stated mechanism was tried to explained.

The rise in viral load and tendency of increased adhesion had a greater correlation. It was also found that platelets which showed increased adhesion was activated following rise in the level of components like selectin, adhesion molecule etc whose function is to aggregate the function of adhesion of thrombocytes.Other mechanism like raised inflammatory reactions that occurs in dengue also causes significant decrease in functional activity of the platelets and also more destruction of the circulating thrombocytes stating that the cause of thrombocytopenia in dengue is multifactorial.

Liver is one of the common organ involved in dengue.The extent of damage that occurs in the organ is shown by the derangement in the liver enzymes. Liver involvement is transient and the liver function return back to normal after recovery from the viral illness the markers of liver damage like ALT,AST has shown to determine severity and adopted in the WHO 2009 classification of dengue.

The host defence mechanism and other various difference between individuals also change the course of the disease to varied extent. Latest studies showed that the gentic makeup of a particular individual also plays significance in the pathogenesis. Research has demonstrated the importance of few genes

"LOC286087, SMAD5, PSPH" etc can lead to that particular individual being more prone to develop the severe form of the disease

In the below diagram various available biomarkers that can be used in predicting and assessing the severity of the dengue infection [34]

Class	Biomarker	
1. Immune activation markers	Cells	Plasmacytoid dendritic cells
		Lymphocytes
		Platelets
	Cytokines	IL-10
		MIF
	Chemokines	CXCL-10
	Complements	C3a, C5a
	Soluble receptors	sCD4/8, sIL-2R, sTNFRII
	Proteases	Tryptase and chymase
2. Endothelial activation markers	Mediators of endothelial	Angiopoietin-1
	function	Angiopoietin-2
	Coagulation pathway	von Willebrand factor
	components	ADAMTS-13
		sThrombomodulin
	Cell surface adhesion molecules	sICAM, sVCAM
	Permeability mediators	VEGF, VEGFRI
		VEGFRII
3. Biochemical markers	Lipids	Total cholesterol, HDL, LDL
	LPS	LPB, CD14
	Liver enzymes	AST, ALT
	Serine protease	IaIp
	Other soluble substances	Nitric oxide
4. Genetic markers	Gene profile	Certain gene expression
	Circulating cell free-DNA	

STRUCTURAL IMPORTANCE OF DENGUE VIRUS

Dengue virus is a positive stranded, encapsulated RNA virus 11kb in size and has a single Open Reading Frame (ORF) encoding for a single polypeptide which is further processed into three structural proteins,(4) that is the Capsid (C), Membrane (M), and Envelope (E) proteins, and seven non-structural (NS1) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5)

Seven nonstructural proteins: NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5. These nonstructural proteins play roles in viral replication and assembly.

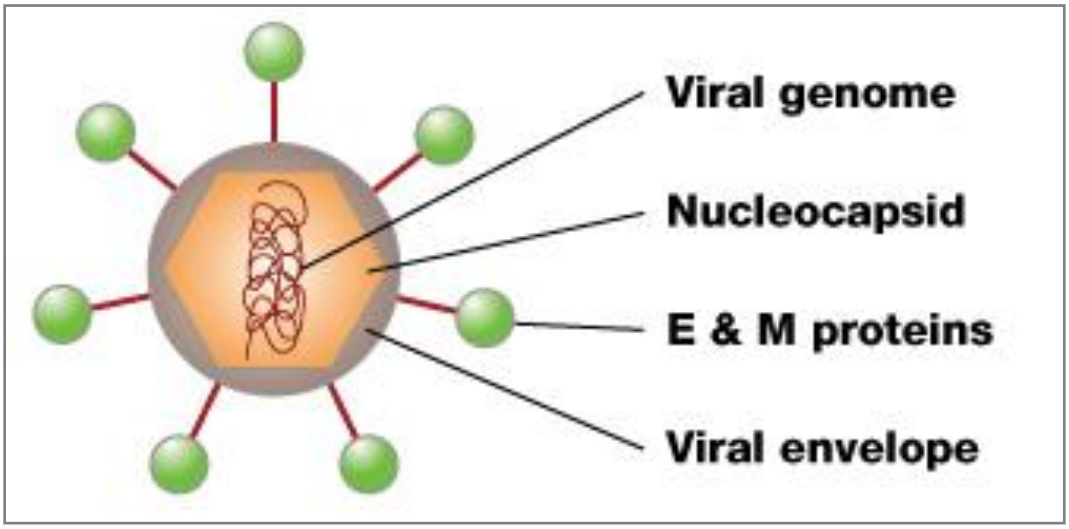


Figure 8 Dengue virus structure

The dengue virus has a roughly spherical shape. Inside the virus is the nucleocapsid, which is made of the viral genome and C proteins. The nucleocapsid is surrounded by a membrane called the viral envelope, a lipid bilayer that is taken

from the host. Embedded in the viral envelope are E and M proteins that span through the lipid bilayer. These proteins form a protective outer layer that controls the entry of the virus into human cells.

The structure of the dengue virus is roughly spherical, with a diameter of approximately 50 nm (1 nm is one millionth of 1 mm) . The core of the virus is the nucleocapsid, a structure that is made of the viral genome along with C proteins. The nucleocapsid is surrounded by a membrane called the viral envelope, a lipid bilayer that is taken from the host. Embedded in the viral envelope are 180 copies of the E and M proteins that span through the lipid bilayer. These proteins form a protective outer layer that controls the entry of the virus into human cells.

Dengue Virus Replication and Infectious Cycle

The dengue viral replication process begins when the virus attaches to a human skin cell . After this attachment, the skin cell's membrane folds around the virus and forms a pouch that seals around the virus particle. This pouch is called an endosome. A cell normally uses endosomes to take in large molecules and particles from outside the cell for nourishment. By hijacking this normal cell process, the dengue virus is able to enter a host cell.

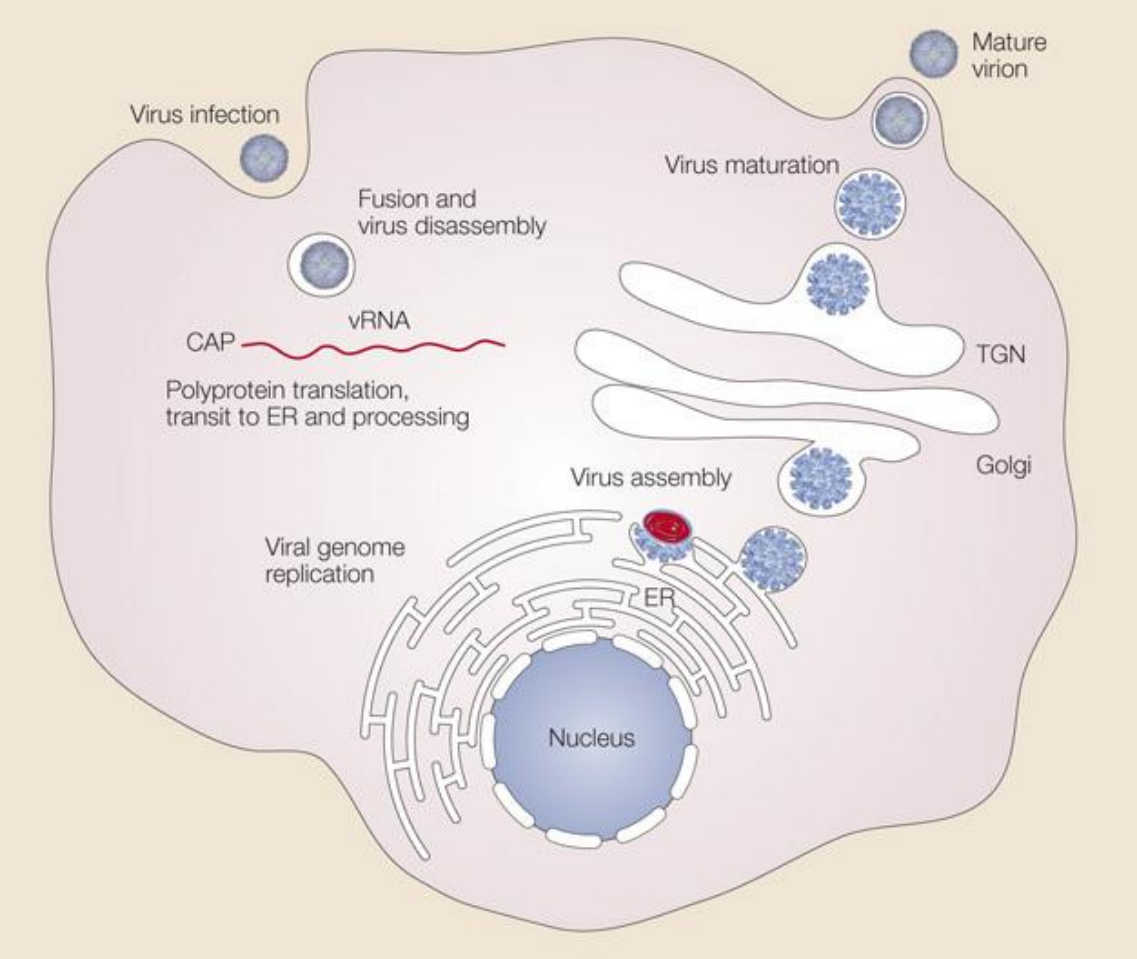


Figure 9: Dengue virus replication

The dengue virus attaches to the surface of a host cell and enters the cell by a process called endocytosis. Once deep inside the cell, the virus fuses with the endosomal membrane and is released into the cytoplasm. The virus particle comes apart, releasing the viral genome. The viral RNA (vRNA) is translated into a single polypeptide that is cut into ten proteins, and the viral genome is replicated. Virus assembly occurs on the surface of the endoplasmic reticulum (ER) when the structural proteins and newly synthesized RNA bud out from the ER. The

immature viral particles are transported through the trans-Golgi network (TGN), where they mature and convert to their infectious form. The mature viruses are then released from the cell and can go on to infect other cells.Once the virus has entered a host cell, the virus penetrates deeper into the cell while still inside the endosome.

1. The endosome must be deep inside the cell where the environment is acidic.
2. The endosomal membrane must gain a negative charge.

These two conditions allow the virus envelope to fuse with the endosomal membrane, and that process releases the dengue nucleocapsid into the cytoplasm of the cell.

Once it is released into the cell cytoplasm, the nucleocapsid opens to uncoat the viral genome. This process releases the viral RNA into the cytoplasm. The viral RNA then hijacks the host cell's machinery to replicate itself. The virus uses ribosomes on the host's rough endoplasmic reticulum (ER) to translate the viral RNA and produce the viral polypeptide. This polypeptide is then cut to form the ten dengue proteins.

The newly synthesized viral RNA is enclosed in the C proteins, forming a nucleocapid. The nucleocapsid enters the rough ER and is enveloped in the ER membrane and surrounded by the M and E proteins. This step adds the viral

envelope and protective outer layer. The immature viruses travel through the Golgi apparatus complex, where the viruses mature and convert into their infectious form. The mature dengue viruses are then released from the cell and can go on to infect other cells.

There are four serotypes of dengue virus (DEN1-4) and the recovery of infection differs from one serotype can confer life-long protection against that serotype but not against the other three serotypes.

Severe dengue infection usually occurs after a second infection with a different serotype, which is due to immune-mediated antibody-dependent enhancement (ADE).

The revised World Health Organization dengue fever guidelines 2011[25] have emphasized the need for early diagnosis and treatment to reduce the mortality due to severe dengue infection.

The classical methods of confirmation of diagnosis are virus isolation, serotype identification, antibody detection tests (IgM and IgG MAC-ELISA), haemagglutination inhibition or neutralization tests but all these tests are time consuming and do not help in the confirmation of diagnosis at an early stage of illness.

The serological diagnosis by IgM/IgG MAC ELISA has been the most common method of confirmation of dengue fever and has sensitivity and specificity of approximately 90% and 98%, respectively but the problem is that it is only detected in the convalescent phase of illness.

With the advent of NS1 antigen assay, there has been marked rise in early diagnosis of dengue fever as it develops during the acute phase of illness (0-7 days) and is emerging as a suitable option for dengue diagnosis in the first week of illness with high sensitivity and specificity.[34]

NS 1 antigen assays show consistently high sensitivity (81%) (32)during first 6 days of illness and is more cost effective. Dengue NS1 antigen is positive for a longer duration in primry dengue infections than in secondary cases.

The use of NS1 rapid diagnostic tests significantly contributes in withholding unnecessary use of antibiotics in patients [34,35]. NS1 antigen positivity can be associated with an increased risk for developing dengue hemorrhgic fever or dengue shock syndrome particularly when it is positive beyond the fifth day of illness which can be used as a warning sign for the severity of dengue fever[34,35]. There are multiple mechanisms by which increased NS1 antigenemia can contribute to severe dengue fever.

NS1 antigen is a 50kDA non structural protein that carries lipids in its secretory form it can contribute to the pathogenesis of severe dengue as lipoproteins are involved in coagulation pathways and can produce vascular inflammation.

NS1 antigen stimulates production of IL 10 and annexin V which are cytokines having an immunosuppressive effect in a dose dependent manner therefore increased levels of circulating NS1 levels can have an indirect immunosuppressive effect contributing to an increased severity of the disease by causing apoptosis of DENV speific T cells[36]

The endothelial glycocalyx layer is a network of proteoglycans and glycoproteins which lines the vascular endothelium and plays an important role in maintaining the endothelial barrier function.It contains sialic acid and heparin sulphate.

Ns1 antigen expresses sialase and heparanase enzymes which causes disruption of endothelial glycocalyx layer and induces vascular hypermaleabilty particularly in microvasculature of lungs leading to capillary leakage and fluid accumulation in third space (pleura) in severe dengue[36,37,38]

Ns1 antigen has also been associated witth stimulation of Macrophage Inhibitory Factor which causes autophagy of endothelial cells leading to vascular leakage and manifestation of severe dengue fever [39,40]

Paranavitane et al [41] evaluated the use of the NS1 antigen test as a marker of severe dengue infection. NS1 antigen positivity especially done within day 5 of illness, was associated with a higher risk of developing severe dengue (odds ratio 3.0).

In order to evaluate the usefulness of the NS1 antigen positivity as a marker of severe clinical disease 313 children with an acute dengue infection was included in the study and there was a significant correlation with liver transaminases.

CLINICALMANIFESTATIONS

Clinical manifestations of dengue disease can lead to a wide range of clinical manifestations, from mild fever to plasma leakage and the potentially fatal dengue shock syndrome.

The clinical manifestations of dengue in infants differ, with a greater incidence of plasma leakage and shock compared with dengue in older children.

Dengue disease was originally classified by the WHO into dengue fever, dengue hemorrhagic fever and dengue shock syndrome.

2011 WHO guidelines classification includes Dengue like illness, Dengue with warning signs and Severe Dengue[25]

Dengue with warning signs

The early signs of the disease are non-specific.

According to the WHO classification (2009) Dengue fever is characterized by febrile episode (≥40 °C for 2–7 days) associated with rash, nausea, vomiting, and headache.

Although the disease affects people of all ages from infancy through to adulthood Studies have showed that children tend to tolerate this phase of illness better than adults

The combination of above mentioned symptoms and other symptoms, such as abdominal pain, mucosal bleed, and lethargy and restlessness can be seen 3–7 days later.

Lab investigations of mild dengue fever cases usually shows abnormal leukocyte counts and moderate elevation of the hepatic amino transferases.

The above symptoms are warning signs to progress for severe dengue if correct management is not done.

Clinical monitoring and surveillance are necessary to prevent vascular leakage, especially in an endemic areas.

Severe dengue

This form of dengue infection can be attributed to any of the four known serotypes DENV 1–4.

About 5–10 % of patients progress to develop a severe Dengue haemorrhagic fever/ Dengue shock syndrome which can be fatal.

This form develops at a late stage of Dengue fever where affected individuals may go through defervescence phase followed by sudden drop of body's temperature.

This phase is also identified by severe bleeding, particularly bleeding from the gastrointestinal tract (black, tarry stool),and thrombocytopenia (<50,000/mm3), which may affect up to 50 % of the affected people .

Interestingly, here was an observed negative correlation between the severity of DHF and the level of platelets in the blood. [39]

The exact mechanism of this correlation is not clear. The drop of platelet counts and the loss of their functionality lead to a vascular fragility increasing the risk of plasma leakage.

CLINICAL COURSE OF DENGUE

WHO divides the course of illness in to three phases -the febrile, critical and recovery phase. Children in the febrile phase typically develop fever, headache with or without retro orbital pain, myalgia, arthralgia, and a maculopapular to petechial rash.

Although children usually suffer from high fever, they are generally less symptomatic during this phase of the illness.

Mild hemorrhagic manifestations like petechiae and mucosal membrane bleeding (e.g. nose and gums) may be seen. This phase lasts for 3-7 days, after which most children recover without complications. In a small proportion of children a systemic vascular leak syndrome becomes apparent around the time of defervescence.

The critical phase is characterized by a progressive leukopenia with a declining platelet count, hemorrhagic manifestations, pleural effusions, ascites and hypoproteinemia.

Shock occurs when a critical volume of plasma is lost through leakage. Vascular leakage and shock are more frequent and more severe in children than in adults while bleeding manifestations and organ involvement are more common in

adults. Clinically significant bleeding in the critical phase of dengue infection in children usually occurs only in association with profound and prolonged shock.

The altered vascular permeability reverts spontaneously to a normal level after approximately 48-72 hours during the recovery phase.

General well-being improves, appetite returns, gastrointestinal symptoms resolves and children start to diurse with normal haemodynamics.

ANTIBODY- RESPONSE IN DENGUE

The antibody response against dengue virus infection is mainly triggered by the precursor membrane (pre -M) and envelope (E) structural proteins and the non -structural protein 1 (NS1) [40-42]

Dengue virions bind to cell surface receptors and virions are internalized by endocytosis.

After release of viral RNA into the cytoplasm of the host cell, the viral genomic RNA is translated to produce viral proteins which assemble into immature virions within the lumen.

In the immature virions, the pre -M protein forms a heterodimer with the E protein. Subsequently, this heterodimer is cleaved which leads to the formation of mature virions, which are secreted from the cell.

In the mature virions, the M protein is completely hidden by the E protein dimers making it inaccessible to antibody binding. However, incomplete cleavage appears to be common, yielding immature particles that can be bound by pre -M protein-specific antibodies. Mature and immature virions induce antibody responses to the E protein[40-42]

SIGNIFICANCE OF NS1 ANTIGEN

The NS1 protein is a glycoprotein that is produced in infected cells, but is not incorporated into the virion. In a child with dengue, NS1 is situated on the plasma membranes of cells and in the circulation.

Antibodies interact with NS1 to cause complement -dependent lysis of virus-infected cells.

Further NS1 - specific antibodies probably contribute to antibody-dependent cellular cyto toxicity.

Neutralization of infection by dengue virus -specific antibodies can occur through several different mechanisms, including inhibition of binding to cell surface receptors or post -binding inhibition of viral fusion with endosomes.

Neutralizing antibodies directed against the E protein are highly serotype cross-reactive.

The tropism of dengue virus for monocytes and macrophages, which both express receptors for immunoglobulins, leads to the entry of dengue virus into host cells,This phenomen is called ‘antibody- dependent enhancement of infection’.

This occurs because virus-antibody complexes infect host monocytes more efficiently than free virus particles.

Anti-body dependent enhancement of infection can be mediated by E protein -specific or pre-M antibodies and occurs when antibody concentration is low so that the number of antibody molecules bound per virion is below the threshold necessary for neutralization of the virus. In genetically predisposed individuals, subsequent infection of monocytes by virus -antibody complexes can also influence cellular immune responses.

NS1 ELISA TESTING

Enzyme-Linked Immunosorbent Assays (ELISA) directed against Non-Structural glycoproteins (NS1 Antigen) have demonstrated very high concentrations in the sera of dengue virus infected patients during the early clinical phase of the disease and represents a new approach to the diagnosis of acute dengue infection.

NS1 antigen assay, there has shown marked rise in early diagnosis of dengue fever during the first week of illness especially during epidemics but its role as an early predictor of severe dengue infection is not very clear.

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 draw backs. The first two assays have restricted scope as a routine diagnostic procedure

Viral isolation by cell culture and subsequent detection by immune fluorescence, though the gold standard cannot be used as a routine diagnostic procedure due to its low sensitivity 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 requirement of paired sera at acute and convalescent phase, which improves the accuracy of the diagnosis, further delays the treatment. NS1 (non-structural protein 1) is a highly conserved glycoprotein that is essential for the

viability of DV and is produced both in membrane-associated and secretory forms by the virus. [43,44,45,46]

Enzyme-linked immunosorbent assays (ELISA) directed against 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.

SIMILAR STUDIES DEMONSTRATING NS1 ANTIGEN TESTING

Study by Kassim et al [47,48] showed the usefulness of the dengue NS1 antigen test was evaluated as a routine, rapid diagnostic test for dengue virus infection. The results reveal the detection rate of dengue virus infection was similar for PCR and dengue antibody (65.9%) and for NS1 antigen and dengue antibody (62.0%) combinations.

Dengue NS1 antigen test can be used to complement the current antibody test used in peripheral laboratories. Thus, the combination of the NS1 antigen and antibody tests could increase the diagnostic efficiency for early diagnosis of dengue infection.

Varen et al [49] suggested NS1 ELISA testing showed sensitivity varies depending on the serological status of the patient, date of specimen collection and serotype of the infecting virus, its use for accurate diagnosis of dengue infection should be considered by clinicians especially early in infection.

A total of 208 sera from patients suspected of having dengue virus infection were collected and tested for dengue antibody, dengue genome and dengue NS1 antigen. Dengue antibody test, dengue PCR test and dengue antigen test were able to detect dengue virus infection from Days 1 to 8 in 72.8, 52.8 and 44.0% of samples, respectively.

Of the 208 sera tested, 69.2% (144/208) of the acute sera were positive for dengue virus infection based on IgM antibody, IgG antibody, NS1 antigen and PCR tests. Thirty-two point two percent of the samples (67/208) were found positive for dengue NS1 antigen, 38.5% (80/208) were PCR positive, 40.9% (85/208) were IgM positive and 36.1% (75/208) were IgG positive for dengue virus

Borez et al conducted[50] a study in 329 patients with age mean group was 6.8 found there is significant increased in platelet count after complete treatment . In severe cases there was done platelet transfusion (18.22 % cases) . 14.92 % cases were admitted in ICU. Diagnosis of dengue by NS1 antigen and IgM Elisa in tertiary care found statistically significant ($P< 0.01$)

Similar study done by Bhazuki et al [51] stated that NS1 ELISA quantified during the early febrile phase and higher sera concenterations were observed in

children with during the early phase of illness was statistically significant in children developing severe dengue. P value observed was <0.05

Thomas et al [52] conducted a study in a tertiary hospital during an outbreak showed that dengue NS1 ELISA titres were found to be high in children during the febrile phase. This study was found to have clinical correlate with severe dengue fever and children were triaged and there was a significant reduction in the mortality.

Study conducted by Alcon et al [53] showed that high circulating levels of NS1 ELISA assay showed a clinical correlation with secondary dengue fever where higher NS1 levels clinically correlated with increasing titres of IgG antibodies done during convasalcent phase. P value was <0.05 and the the study was done in a controlled population who required critical care monitoring.

Jeanne et al [54] suggested a quantitative NS1 ELISA Assay which showed a cut off level of six hundred ng/ml could be used as cutoff level for it so that any individual whose value is more than this within three days of onset of symptoms can be more prone to develop the severe form of the disease especially dengue haemorrhagic fever.

Maria et al [55] showed that high levels of NS1 antigen titres with a cut off of > 50 ng/ml done during first three days of illness was found statistical correlation with children with dengue fever in Indonesian population

Duan et al[56] showed that high levels of NS1 Antigen was also correlated with severe thrombocytopenia and increasing titre values were found to have statistical correlation with shock.[54]

Prospective study done by Dussart et al [57] showed that high circulating levels of NS1 antigen was found to have statistical correlation with children of dengue haemorrhagic fever and the p value was < 0.03

The method using the NS1Ag is one of the very effective method for diagnosis and it has a very good sensitivity level. NS1Antigen is specific to dengue infection , and can detect dengue virus effectively irrespective of the type it belongs. The relative easy availability easy method by which it is performed along with the time factor makes it a very valuable tool for the early diagnosis of the disease and can be used as an early marker during epidemics to classify severe cases.

When it comes to the treatment of patients who are infected with any type of dengue virus care must be taken to closely monitor the patients who are known to

have secondary dengue because patients with secondary infection are vulnerable to severe infections.

MANAGEMENT IN DENGUE

Fluid therapy which restores the third space fluid loss is the most important aspect of treatment. Correct diagnosis with scheduled timing of treatment the burden of the disease can be lowered. Some of the import guidelines proposed are

- Those patients starting to develop the symptoms of dengue but not with any of the warning sign proposed by WHO are to be treated with adequate oral fluids and antipyretic agents and if symptoms are more can also be given adequate analgesics.
- Next if the patient has all the above mentioned features but has developed one or more of the warning signs proposed by WHO then the intensity of oral rehydration therapy should be much more intense and vigorous along with other symptomatic management mentioned above
- If patient has above mentioned features and increase in "PACKED CELL VOLUME-PCV" of less than ten percentage of the previous base value and presence or absence of low platelet count which in this case is between fifty thousand and one lakh patient should be kept

under supervision and other symptomatic measures suggested above should be done

- PCV which has been increased above the level of ten percentage to the previous normal value or base value and if thw patient has presence or absence of the WHO proposed warning signs then the patient should be hydrated well using IV fluids (crystalloids are preferred) and monitoring of the PCV should be done
- If a patient developes shock then care should be given with aggressive colloid infusion and other iv fluids
- If a patient comes under the category of DHF then patient should be given whole blood or FFP based on the amount of bleeding.

Prevention of the disease is very important since it poses a great threat to the community itself rather than a individual or a group of people. Control or preventing the vector which is the major mode responsible for the disease spread is the centre of goal in preventing dengue. Some of the methods are

- Using chemicals to prevent vector development
- Reduction of stagnation of water
- Adequate drainage system
- Mosquito traps

- Preventing the bite using chemical mosquito repellants
- Social awareness about the disease
- Training the medical care system to contain the disease spread during epidemics

NEWER MODALITIES IN DENGUE PREVENTION

Efficient way of preventing dengue infection is Vaccination. The important fact which warrants the necessity for need of a efficient vaccination is that first infection with dengue does not offer permanent immunity and the risk of subsequent infection is also possible. Subsequent reinfection with the dengue virus whith different serotype results in more chance of developing severe form of the disease. This is explained by the inflammatory reaction associated with dengue which plays a important role in the pathogenesis of the disease is increased in subsequent infection.

Developing a vaccine for dengue is difficult because a vaccine should be developed for all of the four existing subtypes. Stephen J [58] has described the various aspects of the vaccination and some of the important trials that is taking place in the field

RESULTS

Our study was conducted at Pediatrics department PSG Hospitals, Coimbatore which is a tertiary care hospital. Our study period was betweenJanuary 2016 to May 2017. In a total of 690 children who were serologically positive for Dengue fever, 270 children were included in the study and 250 children were finally included for study analysis and 20 children were excluded because of logistic reasons and comorbidities. Results were tabulated and comparisons were done.

Table 2

Distribution of study population according to age group

Age group	Number	Percentage
< 5 years	120	48
6-10 years	71	28.4
11-15 years	40	16
>15 years	19	7.6
Total	**250**	**100**

Figure 10 - Distribution of study population according to age group

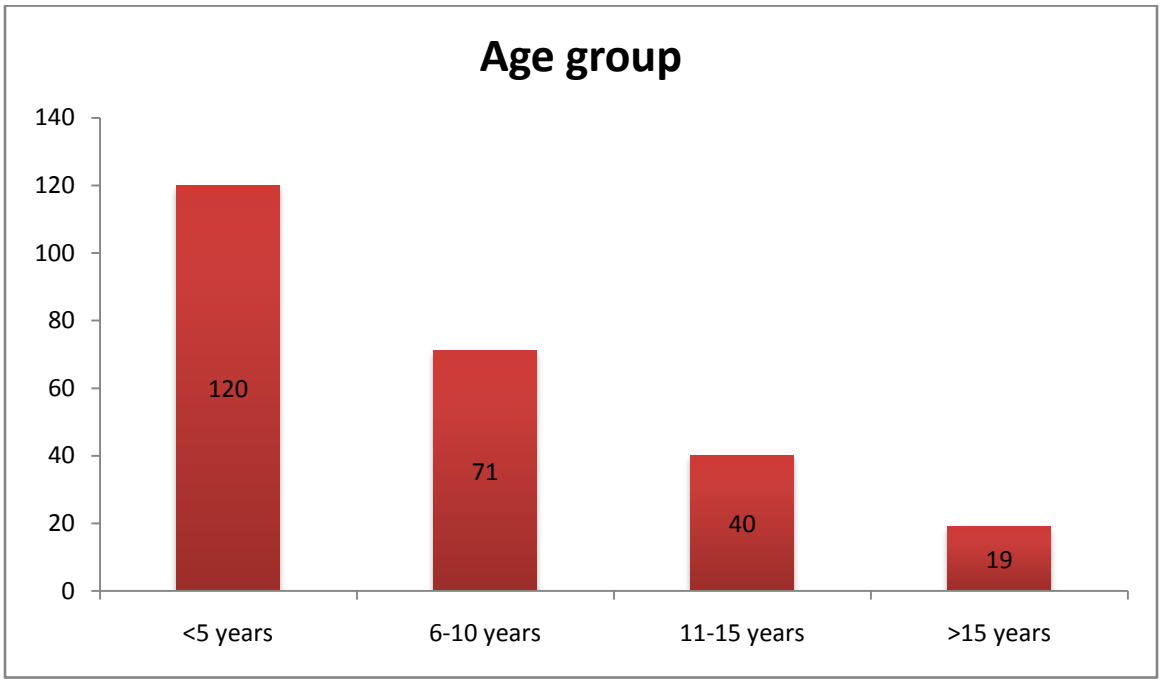


Table 2 and Figure 10 shows the total number of children included in the study. Out of 250 children 120 children (48%) were less than 5 years, 71 children (28.4%) were between 6 to 10 years, 40 children(16%) were between 11 to 15 years and 19 children (7.6%) were above 15 years.

Table 3:

Sex distribution

Study group	Male	Female
Number	133	117
Percentage	53%	47%

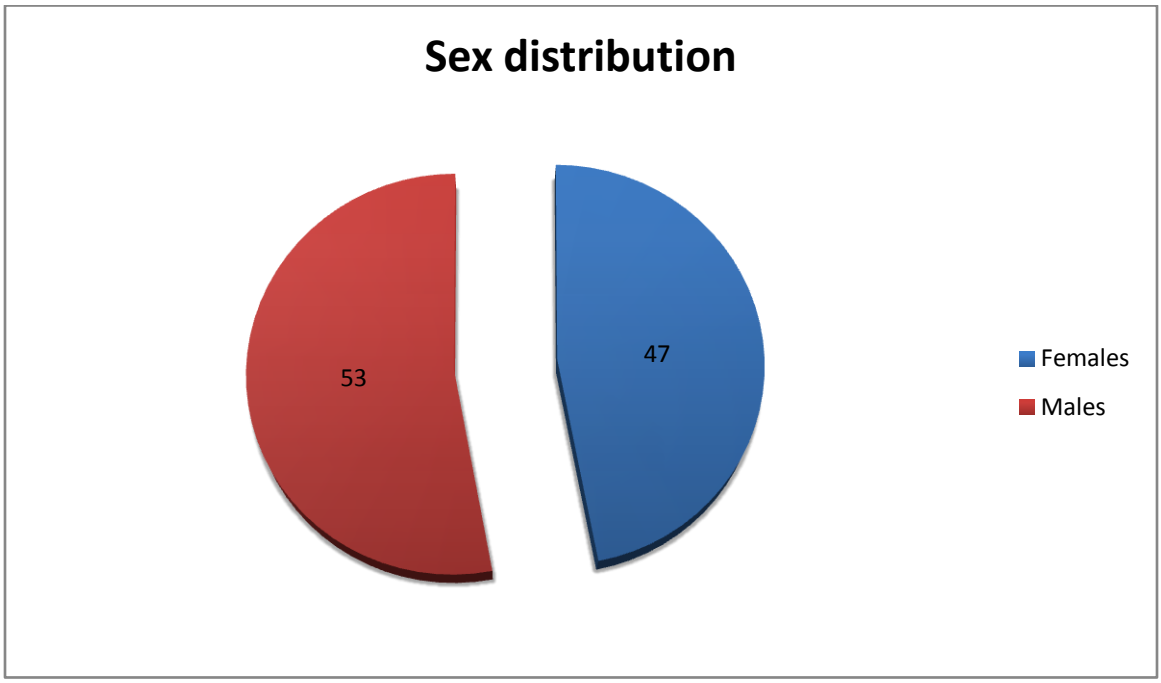


Fig 11 shows the sex distribution of the study group of which 53% were male children and 47% were female children

Table 4

Distribution of study population according to admission status

Admission	Number	Percentage
Admitted	224	89.6
Not admitted	26	10.4
Total	**250**	**100**

Figure 12 Distribution of study population according to admission status

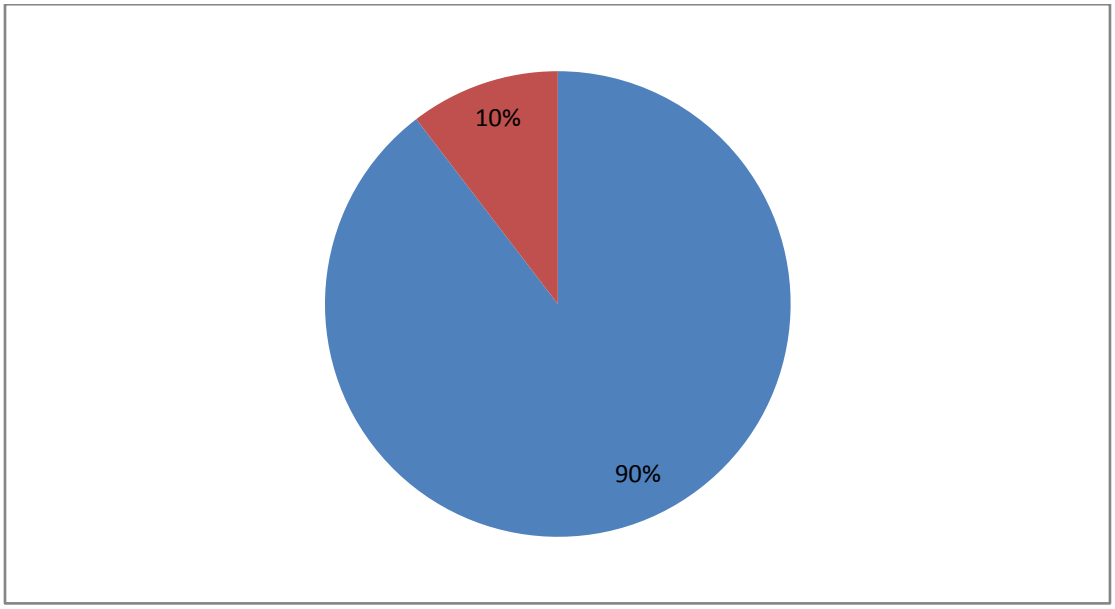


Table 4 and figure 12 shows the admission status of study population. Out of 250 children 224 children were admitted (90%) and 24 children(10%) were treated as outpatients

Table 5

NS1 Antigen positivity related to day of illness

Day of illness	Number	Percentage
≤3 days	158	63.2
4 to 5 days	92	36.8
Total	**250**	**100**

Figure 13- NS1 Antigen positivity related to day of illness

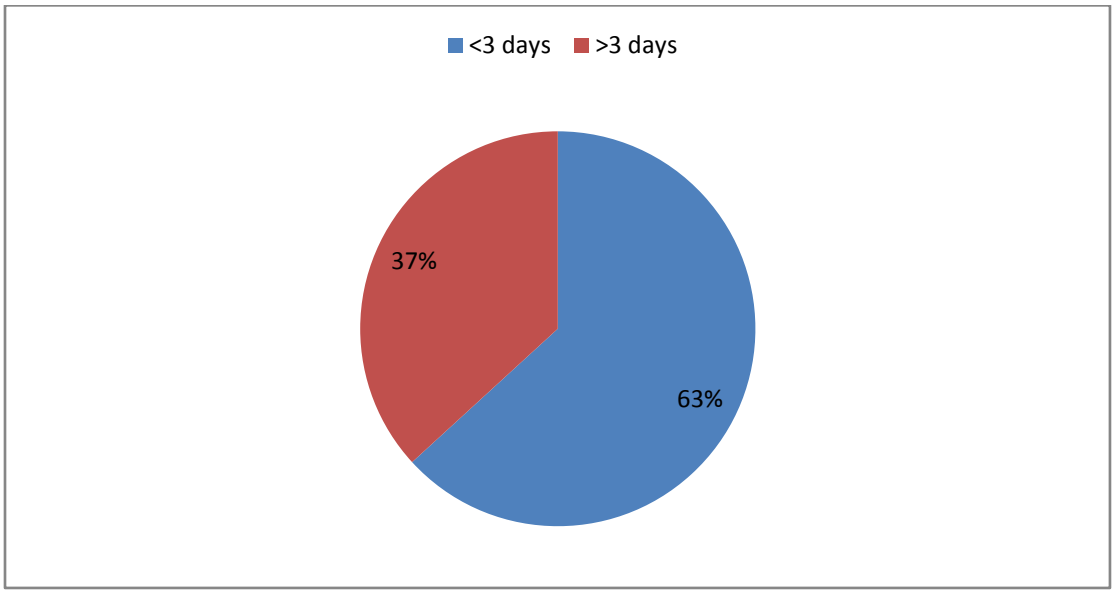


Table 5 and fig 13 shows that NS1 titre values were found to be positive in 158(63.2%) children when NS1 antigen Assay was done in less than 3 days of illness. NS1 antigen was positive in 92 children (36.8%) when done on 4 to 5 days of illness.

Table 6

Distribution of study population according to NS1 titre levels

NS1 levels	Number	Percentage
< 9	81	32.4
9-11	74	29.4
>11	95	38
Total	**250**	**100**

Figure 14 Distribution of study population according to NS1 titre levels

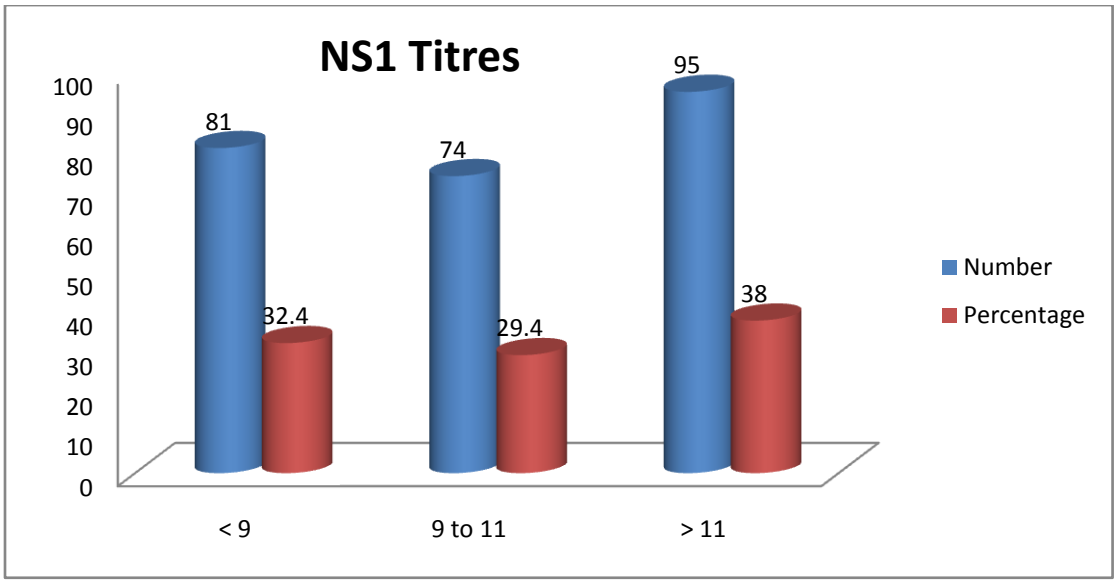


Table 6 and Fig 14 showed the titre values of the study population. Titre values of <9 were seen in 81(32.4%) children, titre values of 9 to 11(29.4%) were seen in 74 children and titre values of >11(38%) were seen in 95(38%)of the children.

Table 7

Association between age group and NS1 titre levels in the study population

Age group	Titres<9	percentage
< 5 years	43	35.8
6 - 10 years	23	32.4
11 - 15 years	8	20
> 15 years	7	36.8

Figure 15 Association between age group and NS1 titre levels in the study population

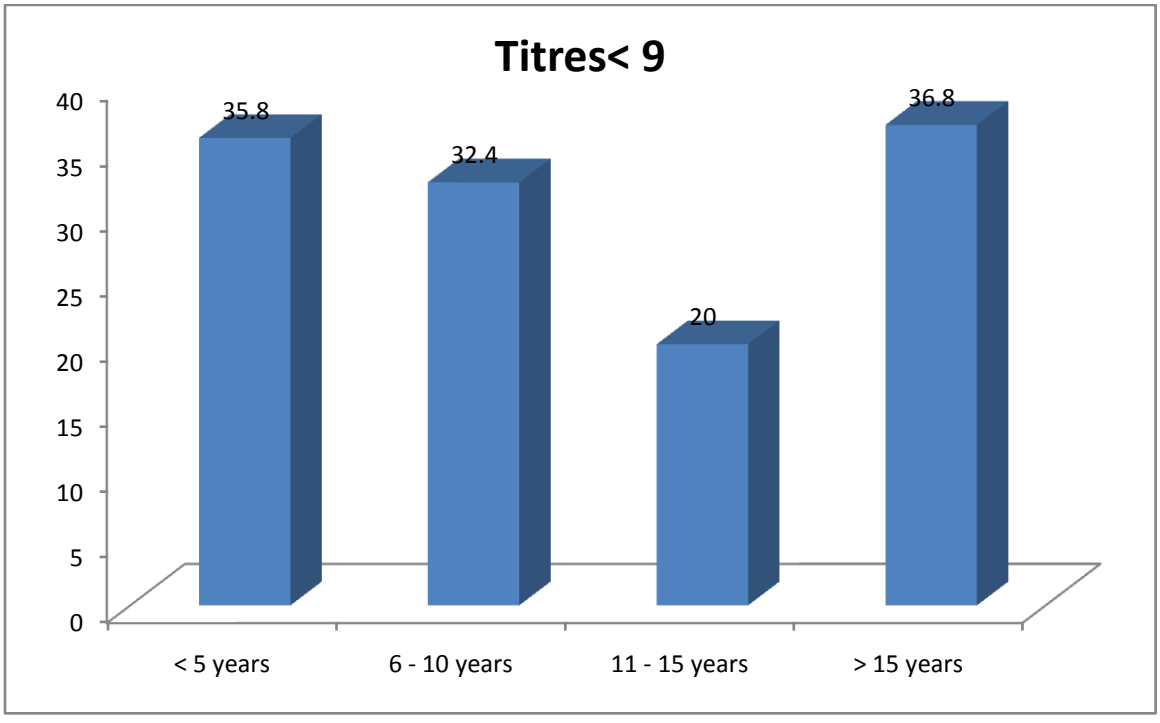


Table 7 and fig 15 shows the association between age group and and NS1 titres. In age group of less than 5 years, titre values of less than9 was seen in 43 children(35.8%)and in age group of 6 to 10 years titre values of less than 9 was seen in 23 children(32.4%). In children with age group of 11 to 15 years titre values of <9 was seen in 8 (20%) children. Children with age group of 11 to 15 years NS1 antigen titre values of <9 was seen in 7(36.8%) children

Table 8 Association between age group and NS1 titre levels in the study population

Age group	Titres 9 to 11	percentage
< 5 years	32	26.7
6 - 10 years	26	36.6
11 - 15 years	12	30
> 15 years	4	21.1

Figure 16 Association between age group and NS1 titre levels in the study population

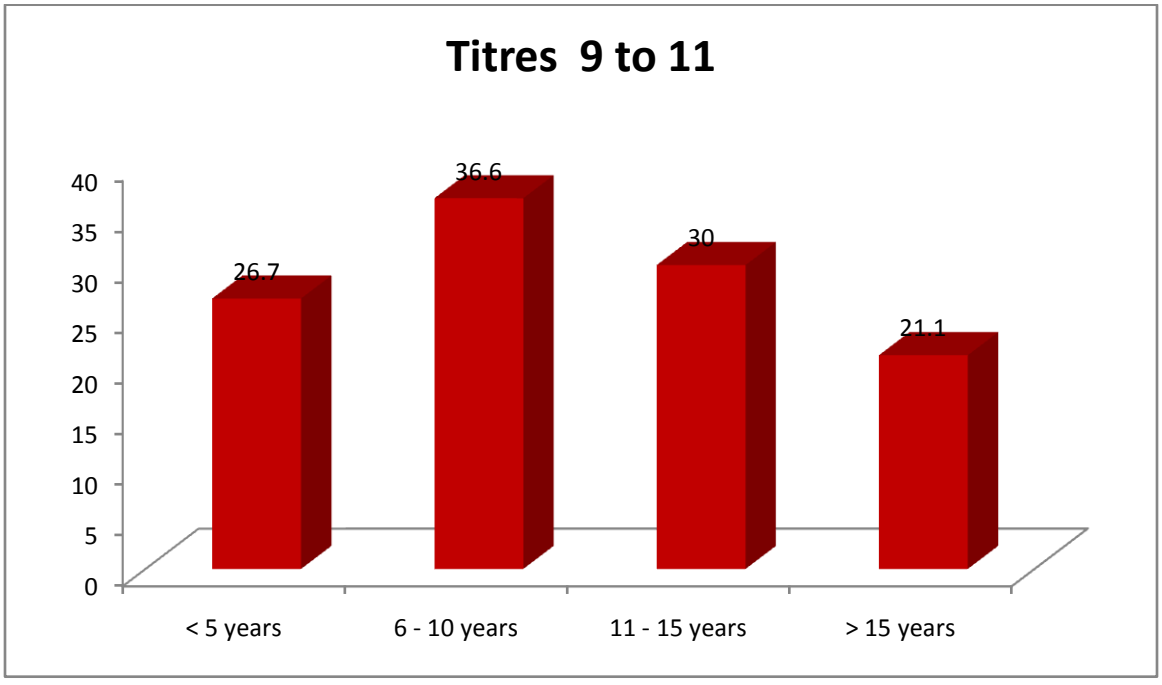


Table 8 and fig 16 showed the association between age group and NS1 titres, In the age group of less than 5 years titre values of 9 to 11 was seen in 32(26.7%)of the children, in the age group of 6 to 10 years titre values of 9 to 11 was seen in 26(36.6%) of the children. In the age group of 11 to 15 years titre values of 9 to 11 was seen in12(30%) of the children, in age group of > 15 years titre values of 9 to 11 was seen in 4 (21.1%) children

Table 9 Association between age group and NS1 titre levels in the study population

Age group	Titres> 11	percentage
< 5 years	45	37.5%
6 - 10 years	22	31%
11 - 15 years	20	50%
> 15 years	8	42.1%

Figure 18 Association between age group and NS1 titre levels in the study population

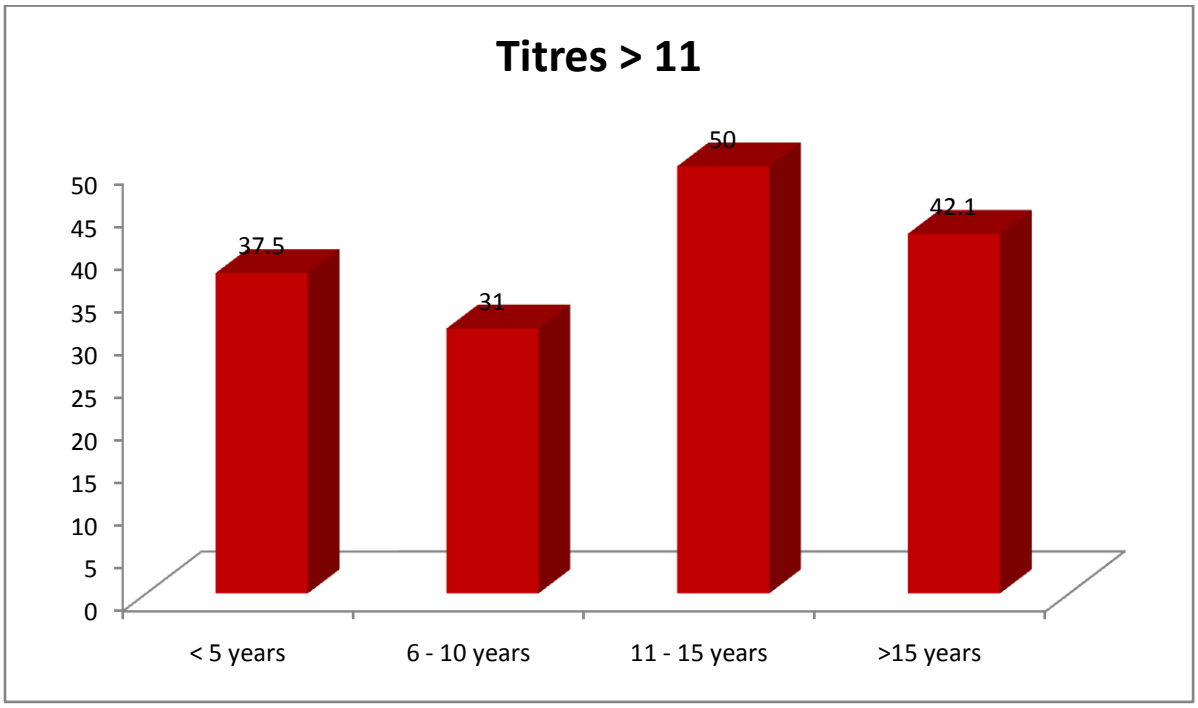


Table 9 and Fig 18 shows the association between age group and NS1 titre levels. In the age group of children <5 years titre values of> 11 was seen in 45(37.5%) children, in children with age group of 6 to 10 years titre values > 11 was seen in 22(31%) of the children. In age group of children between 11 to 15 years titre values of > 11 was seen in 20(50%) of the children. In age group of children > 15 years NS1 titres of > 11 was seen in 8(42.1%) of the children.P value was 0.323 which was not significant.

Table 10

Distribution of study population according to Clinical diagnosis

Clinical diagnosis	Number	Percentage
Mild dengue	128	51.2
Moderate dengue	97	38.8
Severe dengue	25	10
Total	**250**	**100**

Figure 18 Distribution of study population according to Clinical diagnosis

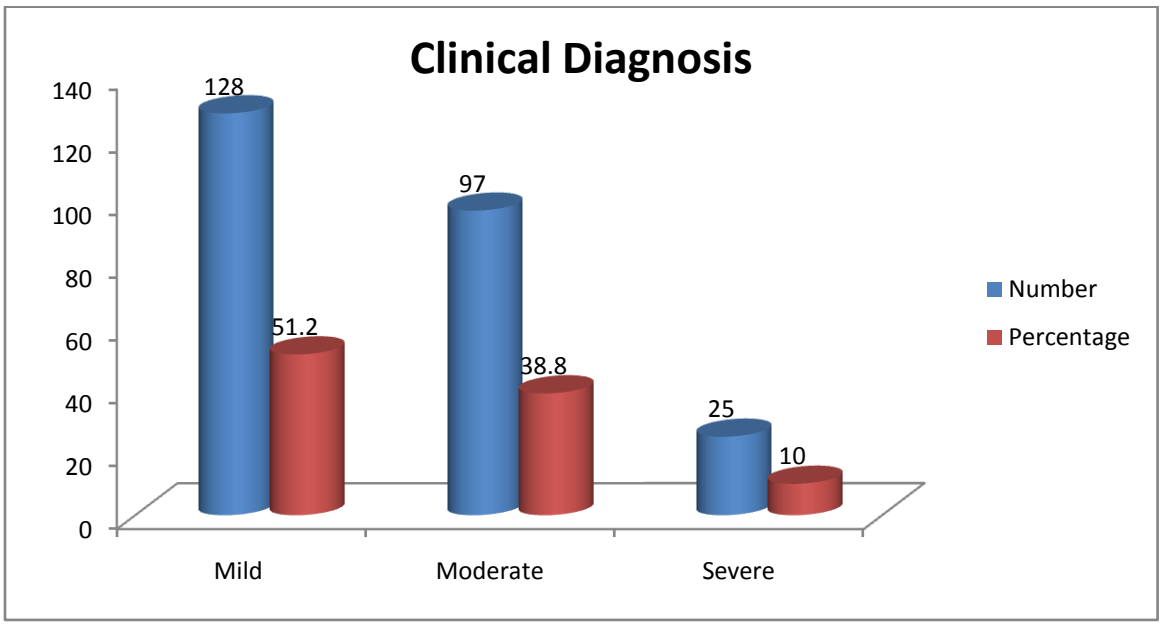


Table 10 and figure 18 shows the distribution of study population according to clinical diagnosis. Out of 250 children Mild dengue was seen in 128(51.2%), Moderate dengue was seen in 97(38.8%) and Severe dengue was seen in 25(10%) of the children.

Table 11

Distribution of study population according to Serology

Type of dengue	Number	Percentage
Primary dengue	122	48.8
Secondary dengue	128	51.2
Total	**250**	**100**

Figure 19 Distribution of study population according to Serology

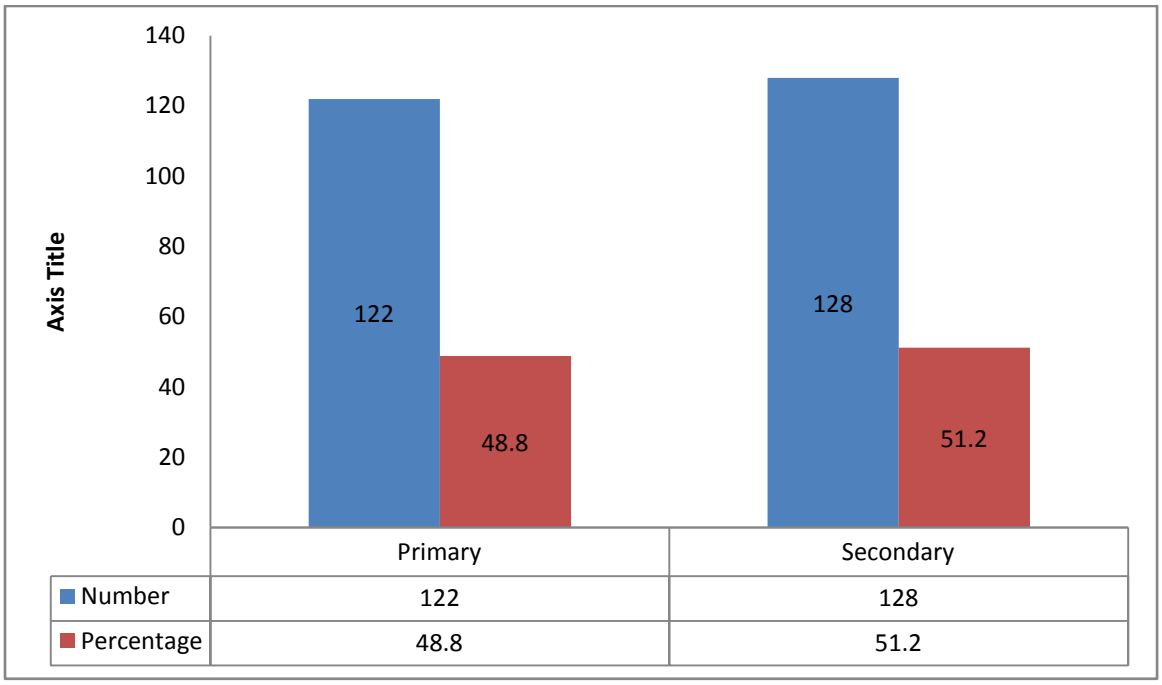


Table 11 and figure 19 showed the distribution of study population according to serology. Out of 250 children 122(48.8%) had primary dengue and 128(51.2%) had secondary dengue fever.

Table 12

Association between age group and clinical diagnosis

	Mild dengue	Moderate dengue	Severe dengue	P value
< 5 years	68(56.7)	40(33.3)	12(10)	0.61(NS)
6-10 years	34(47.9)	30(42.3)	7(9.9)	
11-15 years	16(40)	19(47.5)	5(12.5)	
>15 years	10(52.6)	8(42.1)	1(5.3)	

Figure 20 Association between age group and clinical diagnosis

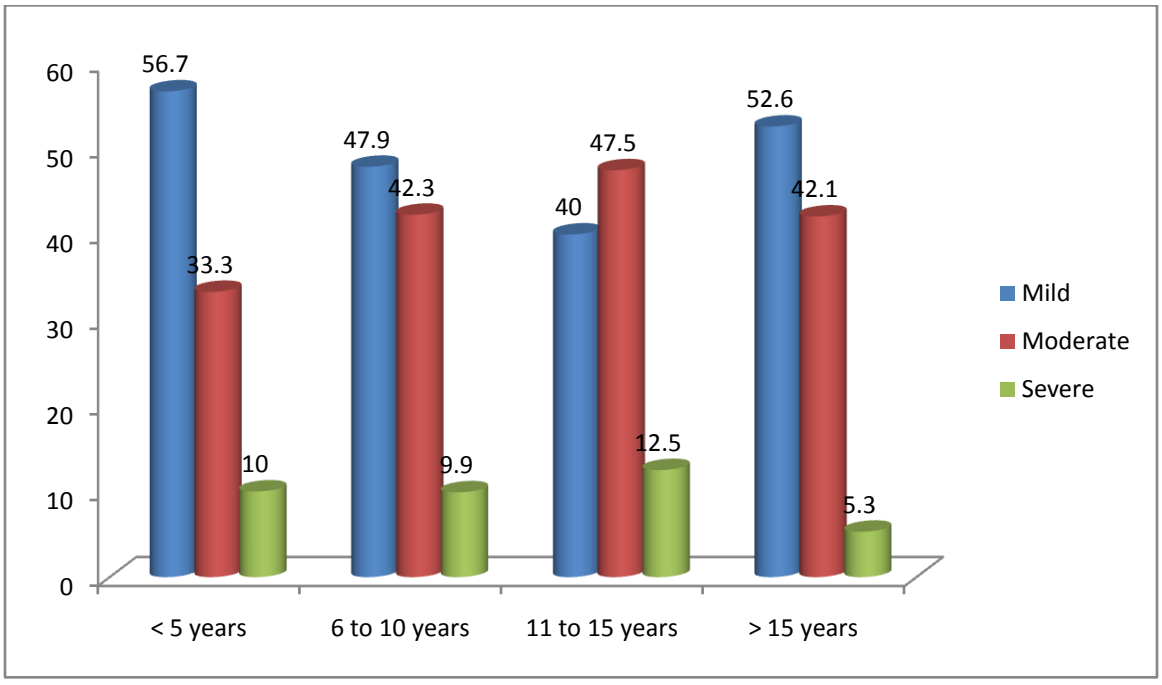


Table 12 and figure 20 showed the association between age group and clinical diagnosis. In age group less than 5 years, Mild dengue was seen in 68(56.7%) of the children, Moderate dengue was seen in 40(33.3%)of the children, Severe dengue was seen in 12(10%) of the children. In the age group of 6 to 10 years, Mild dngue was seen in 34(47.9%) of the children, Moderate dengue was seen in 30(42.3%) of the children, Severe dengue was seen in 7(9.9%) of the children. In the age group of 11 to 15 years , Mild dengue was seen in 16(40%) of the children, Moderate dengue was seen in 19(47.5%) of the children, Severe dengue was seen in 5(12.5%) of the children. In the age group of > 15 years, Mild dengue was seen in 10(52.6%) of the children, Moderate dengue was seen in 8(42.1%) of the children and severe dengue was seen in 1(5.3%)of the children. p value was 0.61 which was not statistically significant.

Table 13

Agreement between NS1 titre levels and clinical diagnosis

NS1 levels	Clinical diagnosis			P value
	Mild dengue	Moderate dengue	Severe dengue	
< 9	42(51.9)	33(40.7)	6(7.4)	0.525
9-11	43(58.1)	27(36.5)	4(5.4)	
>11	43(45.3)	37(38.9)	15(15.8)	

Figure 21 Agreement between NS1 titre levels and clinical diagnosis

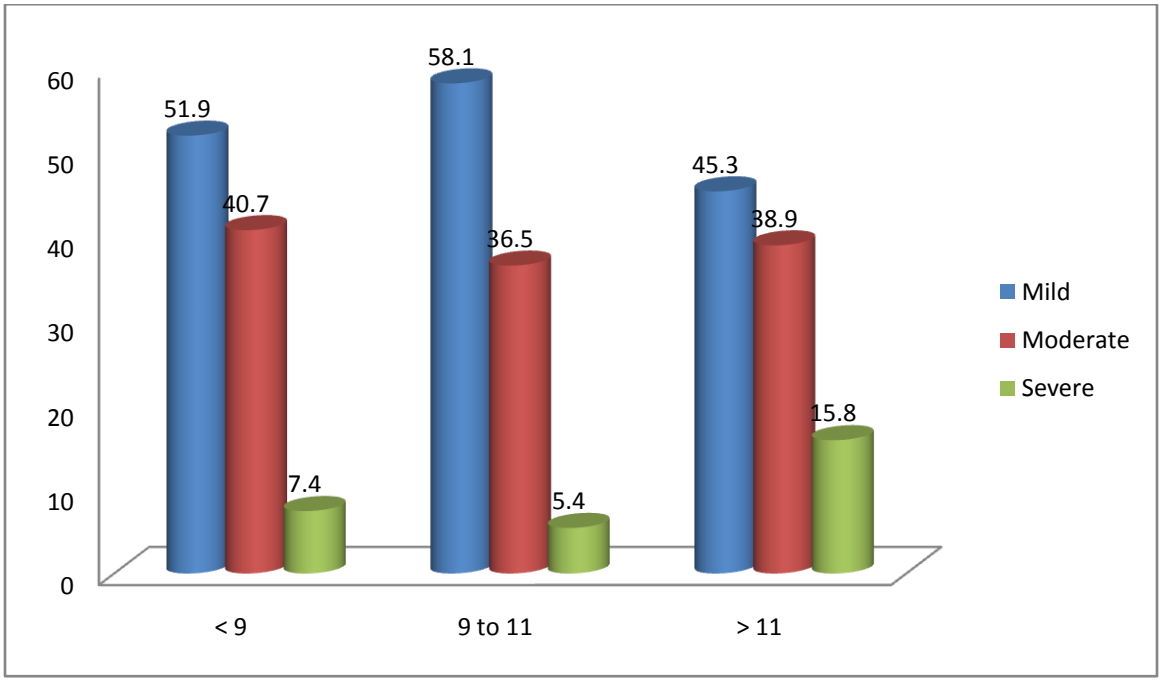


Table 13 and figure 21 shows the Titre levels of <9 was seen in 33(40.7%) of children with moderate dengue, titre values of 9 to 11 was seen in 27(36.5%) of the children diagnosed with moderate dengue, titre values of > 11 was seen in37(38.9%) of children diagnosed with moderate dengue Titre levels of < 9 was seen in 6 children(7.4%) of children diagnosed with severe dengue, titre values of 9 to 11 was seen in 4 (5.4%) children diagnosed with severe dengue, titre values of > 11 was seen in children 15 (15.8%) of children diagnosed with severe dengue. The p value was 0.525 which was not statistically significant.

DISCUSSION

Dengue fever is a recently emerging health problem with increasing epidemics every year. The revised World Health Organization dengue fever guidelines 2011 have stressed the need for early diagnosis and treatment to reduce the mortality due to severe dengue infection.

However there are no proven or clear interventions or modalities to predict the severity of dengue fever in children

In our study we have aimed at developing a predictor of severity of dengue fever.

This is a cross sectional study which was conducted in a tertiary care teaching hospital in Coimbatore, Tamilnadu. We have aimed to find out the association between NS1 Antigen titre levels and the severity of dengue fever in childen between January 2016 to May 2017. NS1 titre levels were measured using ELISA method and it was correlated with Clinical severity.

Demographic distribution of the study

Age

Majority of our study population (48%) were less than 5 years of age and nearly 75% of our study population was less than 10 years. The mean age of children

admitted was 6.8 ± 4.93years. This was similar to Borez et al and Sriram Potha Pregada et al [59] study (the mean age group of children were respectively 6.9 years and 6.8 years.)

Sex distribution

Our study population had slightly male predominance, the male: female ratio were 1.1: 1. This finding was similar to Sriram Potha Pregada et al [59] study

Admission status

Most of our children were treated as inpatients (89.6%) and the remaining 10.4% were treated as out patients. This was contrary to Veasna duong et al[60] study where all the study population were inpatients. The children who were not admitted were mild dengue children without any warning signs.

Observation of NS1 Antigen testing and the day of illness

NS1 Antigen was found to be positive in158(63.2%) children when done in less than 3 days of illness. Ns1 antigen was positive in 92 children (36.8%) when done on 4 to 5 days of illness. Parnavitane et al [41] showed that NS1 Assay was found highly sensitive for dengue infection when done within day 5 of illness and similar observation was found in our study.

Severity of illness

In our study there were 51.2% of mild dengue, 38.2% of moderate dengue and only 10% were severe dengue. This can be attributed to the normal pattern of infectious diseases, where milder illnesses are more common than severe forms of the disease. Similar results were noticed by Alcon et al and Jeanne et al in their studies [53, 54].

Association between study population and serology

Out of 250 children included 122 (48.8%) had primary dengue and 128 (51.2%) had secondary dengue fever.

Association between age group and clinical diagnosis

When the association between age group and clinical diagnosis of mild, moderate and severe dengue was analysed it was observed that in children less than 5 years of age, Mild dengue was seen in 56.7% of the children, Moderate dengue was seen in 40% of the children and 10% of the children had severe dengue.In the age group of children from 6 to 10 years mild dengue was seen in 47.9% of the children, moderate dengue was seen in 42.3% of the children and severe dengue was seen in 9.9% of the children. In children between age group of 11 to 15 years mild dengue was seen in40% of the children, moderate dengue was seen in 47.5% of the children and severe dengue was seen in 12.5% of the children. In children

more than 15 years, mild dengue was seen in 52.6% of the children, 42.1% of the children had moderate dengue and severe dengue was seen in 5.3% of the children. Statistical correlation was done which showed a p value of 0.61 which was not significant. However majority of the children were categorized as mild dengue and the common age of presentation was less than 5 years. Sriram et al [59] did a similar study NS1 Ag was positive in 217 (83.1%) cases and among them non-severe dengue and severe dengue was 143 cases (65.9%) and 74 cases (34.1%) respectively.

Association between age group and ns1 titre values

In the age group of less than 5 years titre values of 9 to 11 was seen in 32(26.7%)of the children, in the age group of 6 to 10 years titre values of 9 to 11 was seen in 26(36.6%) of the children. In the age group of 11 to 15 years titre values of 9 to 11 was seen in12(30%) of the children, in age group of > 15 years titre values of 9 to 11 was seen in 4 (21.1%) children.

In age group of less than 5 years titres titre values of less than9 was seen in 43 children(35.8%) and in age group of 6 to 10 years titre values of less than 9 was seen in 23 children(32.4%). In children with age group of 11 to 15 years titre values of <9 was seen in 8 (20%) children. Children with age group of 11 to 15 years NS1 antigen titre values of <9 was seen in 7(36.8%) children

In the age group of children <5 years titre values of> 11 was seen in 45(37.5%) children, in children with age group of 6 to 10 years titre values > 11 was seen in 22(31%) of the children. In age group of children between 11 to 15 years titre values of > 11 was seen in 20(50%) of the children. In age group of children > 15 years NS1 titres of > 11 was seen in 8(42.1%) of the children. P value was 0.323 which was not statistically significant. Similar observation was seen in study conducted by Dutta et al study [61] where a wide range of pediatric population was included and NS1 Antigen levels with severity of dengue fever did not have any statistical significance. This study was done in teaching hospital in South India which used the similar J Mithra kit for estimation of titres similar to our study.

Association between ns1 titres and clinical diagnosis

When the association between NS1 titre levels and clinical diagnosis was correlated, Titre levels of <9 was seen in 33(40.7%) of children with moderate dengue, titre values of 9 to 11 was seen in 27(36.5%) of the children diagnosed with moderate dengue, titre values of > 11 was seen in37(38.9%) of children diagnosed with moderate dengue. Titre levels of < 9 was seen in 6 children(7.4%) of children diagnosed with severe dengue, titre values of 9 to 11 was seen in 4 (5.4%) children diagnosed with severe dengue, titre values of > 11 was seen in

children 15 (15.8%) of children diagnosed with severe dengue. The p value was 0.525 which was not statistically significant.

Our study observation was similar to Study done by Kosiah et al [62] in Indonesia which showed that NS1 Antigen levels had a poor correlation with severity of dengue fever.

However the above mentioned study was done only in critically ill children whereas in our study we tried to correlate the antigen titres with all children categorised as mild, moderate and severe dengue

Our study semi quantification of NS1 titres was done and titres were not exactly quantitated in contrary to the above mentioned study.

Hence we conclude that NS1 Antigen ELISA titres cannot be used as a predictor of severity in children.

Quantification of NS1 Assays can be recommended which may be warrant early prediction of dengue fever and help in effective management of management.

Further more studies on the utility of NS1 Assay and other interventions may be useful in predicting the severity of dengue fever and to reduce further complications and mortality.

CONCLUSION

In our study we conclude that NS1 Antigen ELISA titres may not be useful in predicting the severity of dengue fever in children and further research is warranted for early identification of severity of dengue which can help in significant reduction in mortality.

LIMITATIONS

However our study had few limitations that NS1 antigen Assay was not quantitated and NS1 Assay was not performed uniformly on one particular day of illness which would have warranted more clinical significance.

BIBILIOGRAPHY

1. Bhat et al, Threat of dengue to blood safety in dengue-endemic countries. Emerg Infect Dis 2009;15:8-11.
2. Martina BE, Koraka P, Osterhaus AD (2009) Common pathogenesis of vector borne diseases, *ClinMicrobiol Rev* 22: 564–581.
3. History of dengue,Epidemiology of dengue: past, present andfuture prospects Annelies Wilder-Smith
4. Evolution of dengue,Natasha Evelyn Anne Murray, 1,2 Mikkel B Quam, and Annelies Wilder-Smith
5. Wang E, Ni H, Xu R, *et al*. Evolutionary Relationship of Endemic/Epidemic and Sylvatic Dengue Viruses. *Journal of Virology* 2000: 74 (7): 3227 – 3234.
6. Journal of south east Tropical health 2011 pages 201-211, cherian et al. epidemics of dengue in India
7. Jounal of Tropical heath 2012, Bhatia et al evolving epidemics of dengue
8. Martina BE, Koraka P, Osterhaus AD (2009) Dengue virus pathogenesis: an integrated view *ClinMicrobiol Rev* 22: 564–581.
9. Leurhaus 2010 et al, integrated approach . *ClinMicrobiol Rev* 25: 599-612.
10. WHO Global strategies of dengue epidemics 2009

11. Azhar EI, Hashem AM, El-Kafrawy SA, Abol-Ela S, Abd-Alla AM, Sohrab SS,et al. Complete genome sequencing and phylogenetic analysis of dengue
12. Joshi et al, Usmani GN, Woda BA, Newburger PE (2013) Advances in understanding the pathogenesis of HLH. Br *J Haematol* 161: 609–622.
13. Martin de Souza VA, Fernandes S, Araujo ES, Tateno AF, Oliveira OM, et al. (2004) Isolation of DENV3 in large Brazilian cohort. *Journal of clinical microbiology* 42: 1782– 1784.
14. Newholam, uruthus.et al,,Advances in understanding the pathogenesis of vertical transmission of dengue, the LANCET 2009*l* 161: 609
15. Fernandes S, Araujo ES, Tateno AF, Oliveira OM, et al. (2004) *Journal of clinical microbiology* 42:Koraka P, Osterhaus AD (2009) Dengue virus pathogenesis: an integrated view. *ClinMicrobiol Rev* 27:676-679
16. Henter JI, Horne A, Arico M, Egeler RM, Filipovich AH, e Diagnostic and pathogenesis of dengue,. Pediatric infectious diseases.
17. Understandingrole of non structural protein in the diagnosis of denguefever,, Pathogeni *st Pract Res Clinmicrobiology* 183–201.
18. Arico M, Egeler RM, Filipovich AH, et al. (2007) pathogenesis of severe dengue fever and various predictors124–131.
19. Studying the complement system in dengue Weiss G (2005) Journal ofclinical immunology rev26: 567-573

20. Thomas L, Najioullah F, Verlaeten O, Martial J, Brichler S, Kaidomar S, et al.Relationship between nonstructural protein 1 detection and plasma viral load in dengue patients. Am J Trop Med Hyg. 2010;83:696-99.
21. Sekaran SD, Lan EC, Mahesawarappa KB, Appanna R, Subramanian G. Evaluation of a dengue NS1 capture ELISA assay for rapid detection of dengue. J Infect Developing Countries. 2007;1:182-88
22. Chaiyaratana W, Chuansumrit A, Atamasirikul K, Tangnararatchakit K (2008) Serum ferritin levels in children with dengue infection. *Southeast Asian J Trop Med Public Health* 39: 832–836
23. World Health Organization (2009) Dengue hemorrhagic fever: diagnosis, treatment, prevention and control (new edition). Geneva.
24. daisy vanitha et a Evaluation of the 2009 WHO dengue case classification in *an Indian pediatric cohort. Am J Trop Med*Hyg 98: 178-190
25. World Health Organization (2009) Dengue hemorrhagic fever: diagnosis, treatment, prevention and control (new edition). Geneva. /Updated WHO guidelines 2011
26. Souverine Mathews et al Guzman MG, Halstead SB, Artsob H, Buchy P, Farrar J, Gubler DJ, Hunsperger E, Kroeger A, Margolis HS, Martinez E, et al: Dengue: a continuing global threat. Nat Rev Microbiol 2010, 8:S7–16.

27. Deaouza et al , Halstead SB: Dengue. Lancet 2007, Studying predictors of severe dengue 370:1644–55.
28. Mathews et al Experimental studies on dengue. I. Isolation, identification and modification of the virus. J Infect Dis 1952, 90:1–9. 5. Gubler DJ: Dengue and dengue hemorrhagic fever. Clin Microbiol Rev 1998, 11:480–96. PMid:9665979 PMCid:88892 6. Ross TM:4.
29. Wang E, Ni H, Xu R, Barrett AD, Watowich SJ, Gubler DJ, Weaver SC: Evolutionary relationships of endemic/epidemic and sylvatic dengue viruses. J Virol 2000, 74:3227–34.
30. Mathews Robin et al Dengue and dengue hemorrhagic fever. Clin Microbiol Rev 1998, 11:480–96.
31. Cornelia ,Barrett AD, Watowich SJ, Gubler DJ, Weaver SC: Evolutionary relationships of endemic/epidemic and sylvatic dengue viruses. J Virol 2000, 74:3227–34.
32. Kala jesi , Guzman MG, Kouri G: Dengue: an update. Lancet Infect Dis 2002, 2:33–42. http://dx.doi.org/10.1016/S1473
33. Wils Juffrie M, van der Meer GM, Hack CE, Haasnoot K, Sutaryo, Veerman AJP, et al. Inflammatory mediators in dengue virus infection in children: Interleukin-8 and its relationship to neutrophil degranulation. Infect Immun. 2000;68(2):702–7

34. Eric Halstead SB, O'Rourke EJ. Dengue viruses and mononuclear phagocytes. I. Infection enhancement by non-neutralizing antibody. J Exp Med. 1977;146:201–17.
35. Krishnamurthy ,Srikiatkhachorn A, Ajariyakhajorn C, Endy TP, Kalayanarooj S, Libraty DH, Green S, et al. Serum interlukin levels is associated with plasma leakage in dengue hemorrhagic fever. J Virol. 2007;81(4):1592–600
36. Guzman MG, Kouri G. Dengue: an update. [2] Lancet Infect Dis. 2002;2:33-42.
37. Chakravarthi A, Kumaria R, Batra VV, Verma V. Improved detection of dengue [3]virus serotypes from serum samples-Evaluation of single-tube multiplex RT-PCR with cell culture. Dengue Bulletin. 2006;30:133-40.
38. Xu H, Pan YX, Qiu LW, Wang YD, Hao W, He LJ, et al. Serotype 1-specific [4] monoclonal antibody-based antigen capture immunoassay for detection of circulating nonstructural protein NS1: implications for early diagnosis and serotyping of dengue virus infections. J Clin Microbiol. 2006;44:2872-78.
39. Thomas L, Najioullah F, Verlaeten O, Martial J, Brichler S, Kaidomar S, et al. Relationship between nonstructural protein 1 detection and plasma viral load in dengue patients. Am J Trop Med Hyg. 2010;83:696-99

40. Sekaran SD, Lan EC, Mahesawarappa KB, Appanna R, Subramanian G. Evaluation of a dengue NS1 capture ELISA assay for rapid detection of dengue and association with thrombocytopenia J Infect Developing Countries. 2007;1:182-88.

41.Pranavitane et al dengue NS1 antigen as a marker of severe clinical disease. BMC Infect Dis. 2014; 14: 570d

41. Susu et al, Usefulness of NS1 a ntigen in the diagnosis of dengue fever.plos 2011,15:78:53-55

42. Shu PY, Huang JH. Current advances in dengue diagnosis. Clinical and Diagnostic Laboratory immunology. 2004;11:642-50.

43. Libraty DH, Young PR, Pickering D, Endy TP, Kalayanarooj S, Green S, et al. High circulating levels of the dengue virus nonstructural protein NS1 early in dengue illness correlate with development of dengue haemorrhagic fever. J Infect Dis 2002;186:1165-8.

44. Kumarasamy V, Wahab AHA, Chua SK, Hassan Z, Chem YK, Mohamed M, Chua KB. Evaluation of a commercial dengue NS1 antigen-capture ELISA for laboratory diagnosis of acute dengue virus infection. J Virol Methods 2007;140:75-9.

45. Bess of K, Delorey M, Sun W, Hunsperger E. Comparison of Two Commercially Available Dengue Virus (DENV) NS1 Capture Enzyme-

Linked Immuno sorbent Assays Using a Single Clinical Sample for Diagnosis of Acute DENV Infection. Clin Vaccine Immunol 2008;15:1513-8.

46. Schilling S, Ludolfs D, Le VA, Schmitz H. Laboratory diagnosis of Primary and secondary dengue infections. J Clin Virol 2004;31:179-84.
47. Ampaiwan C, Wathanee C, Viroj P, Kanchana T, Sarapee L, Sutee Y. The use of Nonstructural protein 1 antigen for the early diagnosis during the febrile stage in patients with dengue infection. J Paed Infect Dis 2008;27:43-8.
48. Southeast Asian J Trop Med Public Health. 2011 May;42(3):562-9.Use of dengue NS1 antigen for early diagnosis of dengue virus infection.Kassim FM, Izati MN, TgRogayah TA, Apandi YM, Saat Z.
49. Varen Evaluation of a Dengue NS1 Antigen Detection Assay Sensitivity and Specificity for the Diagnosis of Acute Dengue Virus Infectio NVARENL LaurHerm Butsaya Thaisomboonsuk,Yongyuth Poolpanichupatam, Richard Jarman,Siripen Kalayanarooj,Ananda Nisalak,In-Kyu Yoon,and Stefan Fernandez
50. Borez Young PR, Hilditch PA, Blectchy C, Halloran W. An antigen capture enzyme linked immunosorbent assay reveals high levels of dengue virus

protein NS1 in the sera of infected patients. J Clin Microbiol. 2000;38:1053 to57

51. Bhazuki,Xu H, Pan YX, Qiu LW, Wang YD, Hao W, He LJ, et al. Serotype 1-specific [14]monoclonal antibody-based antigen capture immunoassay for detection of circulating nonstructural protein NS1: implications for early diagnosis and serotyping of dengue virus infections. J Clin Microbiol. 2006;44:2872-78.
52. ThomasHenchal EA, McCown JM, Sequin MC, Gentry MK, Brandt WE. Rapid identification of dengue virus isolates by using monoclonal antibodies in an indirect immunoflourescent assay. Am J Trop Med Hyg. 1983;32:164-69.
53. Alcon S, Talarmin A, Debruyne M, Falconar A, Duebel V, Flammand M. Enzyme linked immunosorbent assay specific to dengue virus type 1 nonstructural protein ns1 reveals circulation of the antigen in the blood during acute phase of the disease in patients experiencing primary or secondaryinfections. J Clin Microbiol. 2002;40:376-81.
54. Jeanne Budiyanto A, Puspitasari D, Husada D, Darmowandowo W, [18] Ismoedijanto, et al. Application of revised dengue classification criteria as a severity marker of dengue viral infection in Indonesia. Southeast Asian J Trop Med Public Health. 2010;41:1088-94.

55. Maria Ismoedijanto, et al. Application of revised dengue classification criteria as a severity marker of dengue viral infection in Indonesia. Southeast Asian J Trop Med Public Health. 2010;41:1088-94

56. Duan et al Kittigul L, Pitakarnjanakul P, Sujirarat D, Siripanichgon K. The differences of clinical manifestations and laboratory findings in children and adults with dengue virus infection. J Clin Virol. 2007;39(2):76-81.

57. Dussart P, Labeau B, Lagathu G, Louis P, Nunes MRT, Rodrigues SG, et al. Evaluation of an Enzyme Immunoassay for detection of dengue virus NS1 antigen in human serum. Clin Vaccine Immunol. 2006;13:1185-89

58. Stephen J. ThomasDavid W. Vaughn· Newer vaccines effective against DENV1-3 in Journal of infectious diseases

59. Sriram PothaPregada, BanuPriya, KamalaKannan2 mahalaKShmy thulaSingam. Journal of Clinical and Diagnostic Research. 2016 Apr, Vol-10(4)

60. Vaesana Lapphra K, Sangcharaswichai A, Chokephaibulkit K, Tiengrim S, Piriyakarnsakul W, et al. (2008) Evaluation of an NS1 antigen detection for diagnosis of acute dengue infection in patients with acute febrile illness in an Indonesian cohort. Diagn Microbiol Infect Dis 60: 387–391

61. Datta W, Wattal C. Dengue NS1 antigen detection test: A useful tool in early [10] diagnosis of dengue virus infection. Indian J Med Microbiol. 2010;28:107-10

62. Kosiah Duong V, Ly S, Lorn Try P, Tuiskunen A, Ong S, et al. Evaluation of a Dengue NS1 Antigen Detection Assay Sensitivity and Specificity for the Diagnosis of Acute Dengue Virus Infection PLoS Negl Trop Dis 5: e1244.

PSG Institute of Medical Sciences & Research
Institutional Human Ethics Committee

Recognized by The Strategic Initiative for Developing Capacity in Ethical Review (SIDCER)
POST BOX NO. 1674, PEELAMEDU, COIMBATORE 641 004, TAMIL NADU, INDIA
Phone : 91 422 - 2598822, 2570170, Fax : 91 422 - 2594400, Email : ihec@psgimsr.ac.in

July 3, 2017

To
Dr M Lavanya
Postgraduate
Department of Paediatrics
PSG IMS & R
Coimbatore
Guide/s: Dr A Jayavardhana / Dr B Appalaraju

The Institutional Human Ethics Committee PSG IMS & R, Coimbatore - 4, has reviewed your proposal on 30th June, 2017 in its expedited review meeting held at IHEC Secretariat, PSG IMS&R, between 10.00 am and 11.00 am, and discussed your request for approval for waiver of consent for the study entitled:

"Correlation of NS1 antigen assay with severity and outcome of dengue fever in children"

The following documents were received for review:

1. Your letter dated 24.06.2017
2. Amendment reporting form dated 24.06.2017
3. Application for waiver of consent

After due consideration, the Committee has decided to approve you request for waiver of consent for the above study.

The members who attended the meeting held on at which your proposal was discussed, are listed below:

Sl. No.	Name of the Member of IHEC	Qualification	Area of Expertise	Gender	Affiliation to the Institution Yes/No	Present at the meeting Yes/No
1	Mr R Nandakumar (Chairperson, IHEC)	BA., BL	Legal Expert	Male	No	Yes
2	Dr. S. Bhuvaneshwari (Member-Secretary, IHEC)	MD	Clinical Pharmacology	Female	Yes	Yes
3	Dr S Shanthakumari	MD	Pathology, Ethicist	Female	Yes	Yes
4	Dr Sudha Ramalingam	MD	Epidemiologist, Ethicist Alt. member-Secretary	Female	Yes	Yes
5	Dr D Vijaya	M Sc., Ph D	Basic Medical Sciences (Biochemistry)	Female	Yes	Yes

This Ethics Committee is organized and operates according to Good Clinical Practice and Schedule Y requirements.

Non-adherence to the Standard Operating Procedures (SOP) of the Institutional Human Ethics Committee (IHEC) and national and international ethical guidelines shall result in withdrawal of approval (suspension or termination of the study). SOP will be revised from time to time and revisions are applicable prospectively to ongoing studies approved prior to such revisions.

Kindly note this approval is subject to ratification in the forthcoming full board review meeting of the IHEC.

Yours truly,

SECRETARY
PSG IMS&R

Dr S Bhuvaneshwari
Member – Secretary
Institutional Human Ethics Committee
Proposal No. 15/437

PSG Institute of Medical Science and Research, Coimbatore
Institutional Human Ethics Committee
INFORMED CONSENT FORMAT FOR RESEARCH PROJECTS

(strike off items that are not applicable)

I **Lavanya M., Jayavardhana A., Appalu Raju**, am / a carrying out a study on the topic: Correlation of NS1 antigen assay with the severity and outcome of dengue fever in children

as part of my / research project being carried out under the aegis of the Department of: Peadiatrics

(*Applicable to students only*): My research guide is: DR. A.JAYAVARDHANA

The justification for this study is: This study is done to justify the utility of NS 1 antigen in the management of fever in children in a resource limited set up

The objectives of this study are:

Primary Objective: To assess the correlation between NS1 antigen and severity of the disease and outcome in children

Secondary Objective: To find the correlation between NS 1 antigen and severity of dengue fever

Sample size: 100.

Study volunteers / participants are (specify population group & age group): ____Children diagnosed as dengue fever less than 18 years of age_____.

Location: ____PSG hospitals, Coimbatore_____.

We request you to kindly cooperate with us in this study. We propose collect background information and other relevant details related to this study. We will be carrying out:

Initial interview (specify approximate duration):____5_____ minutes.

Data collected will be stored for a period of ____3_ years. We will / will not use the data as part of another study.

Health education sessions: Number of sessions: _____. Approximate **duration** of each session:
_____ minutes.

Clinical examination (Specify details and purpose):

Blood sample collection: Specify quantity of blood being drawn: _____2_____ml.

No. of times it will be collected: _____1_____.

Whether blood sample collection is part of routine procedure or for research (study) purpose:

- ✓ 1. Routine procedure
- ✓ 2. Research purpose

Specify **purpose**, discomfort likely to be felt and side effects, if any: _____

Whether blood sample collected will be stored after study period: Yes / **No**, it will be destroyed

Whether blood sample collected will be sold: Yes / **No**

Whether blood sample collected will be shared with persons from another institution: Yes / **No**

Medication given, if any, duration, side effects, purpose, benefits:

Whether medication given is part of routine procedure: Yes / No (If not, state reasons for giving this medication)

Whether alternatives are available for medication given: Yes / No (If not, state reasons for giving this particular medication)

Final interview (specify approximate duration):____5_____ mts. If **photograph** is taken, purpose:

Benefits from this study:

Risks involved by participating in this study: Nil

How the **results** will be used: For the interpretation of NS1 antigen assay with the severity of the disease in children

If you are uncomfortable in answering any of our questions during the course of the interview / biological sample collection, **you have the right to withdraw from the interview / study at anytime.** You have the freedom to withdraw from the study at any point of time. Kindly be assured that your refusal to participate or withdrawal at any stage, if you so decide, will not result in any form of compromise or discrimination in the services offered nor would it attract any penalty. You will continue to have access to the regular services offered to a patient. You will **NOT** be paid any remuneration for the time you spend with us for this interview / study. The information provided by you will be kept in strict confidence. Under no circumstances shall we reveal the identity of the respondent or their families to anyone. The information that we collect shall be used for approved research purposes only. You will be informed about any significant new findings - including adverse events, if any, – whether directly related to you or to other participants of this study, developed during the course of this research which may relate to your willingness to continue participation.

Consent: The above information regarding the study, has been read by me/ read to me, and has been explained to me by the investigator/s. Having understood the same, I hereby give my consent to them to interview me. I am affixing my signature / left thumb impression to indicate my consent and willingness to participate in this study (i.e., willingly abide by the project requirements).

Signature / Left thumb impression of the Study Volunteer / Legal Representative:

Signature of the Interviewer with date: Witness:

Contact number of PI:

Contact number of Ethics Committee Office: 0422 2570170 Extn.: 5818

SOP 03-V 3.0 / ANX 10-V 3.0

Institutional Human Ethics Committee
PSG Institute of Medical Sciences and Research, Coimbatore

Parental Consent Form

Title of Study:Correlation of NS1 antigen assay with the severity and outcome of dengue fever in children.

Name of the Principal Investigator: Dr. Lavanya M

Department:
Your (son/daughter/child/infant/adolescent youth) is invited to participate in a study of (describe the study).

My name is Dr.Lavanya.Mand I am a junior resident at PSG Institute of Medical Sciences and Research, Coimbatore. This study is (state how study relates to your program of work or your supervisor's program of work).

I am asking for permission to include your (son/daughter/child/infant/adolescent youth) in this study because

I expect to have 100 (Number) participants in the study.
If you allow your child to participate, (state who will actually conduct the research) will (describe the procedures to be followed.)

Any information that is obtained in connection with this study and that can be identified with your (son/daughter/child/infant/adolescent youth) will remain confidential and will be disclosed only with your permission. His or her responses will not be linked to his or her name or your name in any written or verbal report of this research project.

Your decision to allow your (son/daughter/child/infant/adolescent youth) to participate will not affect your or his or her present or future relationship with PSGIMS&R or PSG Hospitals or (include the name of any other institution connected with this project). If you have any questions about the study, please ask me. If you have any questions later, call me at 08012017880. If you have any questions or concerns about your (son/daughter/child/infant/adolescent youth)'s participation in this study, call……..

You may keep a copy of this consent form.

You are making a decision about allowing your (son/daughter/child/infant/adolescent youth) to participate in this study. Your signature below indicates that you have read the information provided above and have decided to allow him or her to participate in the study. If you later decide that you wish to withdraw your permission for your (son/daughter /child/ infant/adolescent youth) to participate in the study, simply tell me.

You may discontinue his or her participation at any time. *This will not affect in any way your future treatment in this hospital.*

Printed Name of (son/daughter/child/infant/adolescent youth)

Signature of Parent(s) or Legal Guardian with Date Signature of Investigator with Date

மனித நெறிமுறைக் குழு, பூ சா கோ மருத்துவக் கல்லூரி மற்றும் ஆராய்ச்சி நிறுவனம்

ஆராய்ச்சியில் பங்கு பெறுவதற்கான ஒப்புதல் படிவம்
7 முதல் 18 வயதிற்கு உட்பட்ட குழந்தைகளுக்கானது

நாங்கள் எதற்காக உங்களை சந்திக்கிறோம்?

நாங்கள் மேற்கொள்ள இருக்கும் ஆய்வினைப் பற்றி உங்களுக்கு கூற உள்ளோம். ஆய்வு என்பது மருத்துவர்கள் உடல் நலம் மற்றும் வியாதிகள் குறித்து தகவல்கள் சேகரித்தல் ஆகும். மருத்துவர் **லாவன்யா .போ,** மற்றும் சிலர் சேர்ந்து நகர்ப்புறம் மற்றும் **"குழந்தைகளுக்கு ஏற்படும் தீவிர டெங்குக் காய்சலுக்கும் இரத்தத்தில் உள்ள NS1 ஆன்டிஜனின் அளவுகளுக்கும் உள்ள சம்பந்தம் பற்றிய ஆய்வு"** பற்றி மேலும் தகவல்கள் அறிந்து கொள்ள ஒரு ஆய்வு மேற்கொள்ள உள்ளனர். அதனைப் பற்றி நாங்கள் உங்களுக்கு விவரமாகக் கூறிய பின் இந்த ஆய்வில் பங்கேற்பது பற்றி உங்கள் கருத்தினைத் தெரிவிக்கலாம்.

நாங்கள் எதற்காக இந்த ஆய்வினை மேற்கொள்கிறோம்?

நாங்கள் **தீவிர டெங்கு காய்ச்சலை விரைவாக இரத்தத்தில் NS1 ஆன்டிஜனின் மூலம்** கண்டுபிடிக்க முயற்சிக்கிறோம். எனவே உங்களைப் போன்ற பல குழந்தைகளிடம் தகவல் சேகரிக்க உள்ளோம். இந்த ஆய்வில் சுமார் குழந்தைகள் பங்கு பெற உள்ளனர்.

இந்த ஆய்வில் நீங்கள் பங்கேற்றால் என்ன நடக்கும்?

1. உங்கள் கையிலிருந்து 1 முறை ஊசி மூலம் இரத்தம் எடுக்கப்படும்.

இந்த ஆய்வினால் வலி ஏற்படுமா?

ஊசி குத்தும் பொழுது சிறிது வலி ஏற்படும். அது விரைவில் சரியாகி விடும்.

இந்த ஆய்வில் பங்கேற்பதால் நலம் அடைவீர்களா?

இல்லை. இதனால் நீங்கள் நலமடைய முடியாது. ஆனால், மருத்துவர்கள் இந்த ஆய்வின் மூலம் பிற்காலத்தில் உங்களைப் போன்ற குழந்தைகளுக்கு உபயோகப் படும் வகையில் சில வழிமுறைகளைக் கண்டுபிடிக்கலாம்.

எல்லோருக்கும் என் நிலை பற்றி தெரிய வருமா? (நம்பகத்தன்மை)

நீங்கள் இந்த ஆய்வில் பங்கேற்பதை நாங்கள் மற்றவர்களுக்குத் தெரிவிக்க மாட்டோம். உங்களைப் பற்றிய தகவல்களை ஆய்வில் சம்பந்தப் படாத நபர்களுக்குத் தெரிவிக்க மாட்டோம்.

இந்த ஆய்வு எனக்கு கெடுதல் அல்லது ஆபத்து விளைவிக்குமா?

இல்லை

இந்த ஆய்வில் பங்கேற்பதால் எனக்கு எதுவும் கிடைக்குமா?

இல்லை

நீங்கள் எனக்கு இந்த ஆய்வின் முடிவுகளைத் தெரிவிப்பீர்களா?

இல்லை

உங்களுக்கு ஏதேனும் கேள்விகள் உள்ளதா?

நீங்கள் இப்பொழுதோ பின்னரோ எந்த நேரத்திலும் உங்கள் சந்தேகங்களைக் கேட்கலாம். என்னிடமோ அல்லது இந்த ஆய்வின் பிற ஆராய்ச்சியாளர்களிடமோ உங்கள் சந்தேகங்களைக் கேட்கலாம்.

இந்த ஆய்வில் நீங்கள் பங்கேற்க வேண்டுமா?

இல்லை. இதில் பங்கேற்காததால் உங்களை யாரும் எதுவும் செய்யப்போவதில்லை. உங்களுக்கு விருப்பம் இல்லை என்றால் எங்களிடம் தெரிவிக்கலாம். நீங்கள் பங்கேற்க விரும்பினாலும் எங்களிடம் தெரிவிக்கலாம். நீங்கள் இப்பொழுது ஒத்துக் கொண்டு பின்னர் மனம் மாறி ஆய்விலிருந்து விலகுவதானால் விலகலாம் என்பதையும் மனதில் கொள்ளவும். இது நீங்கள் பிற்காலத்தில் மருத்துவமனியில் பெறும் சிகிச்சையை எந்த விதத்திலும் பாதிக்காது என்பதையும் நாங்கள் உங்களுக்கு தெரிவித்துக்கொள்ள விரும்புகிறோம்.

நான் யாரிடம் பேச அல்லது சந்தேகம் கேட்க முடியும்?

நீங்கள் இப்பொழுதோ பின்னரோ எந்த நேரத்திலும் உங்கள் சந்தேகங்களைக் கேட்கலாம். என்னிடமோ அல்லது இந்த ஆய்வின் பிற ஆராய்ச்சியாளர்களிடமோ உங்கள் சந்தேகங்களைக் கேட்கலாம்.

உங்களுக்கு விருப்பம் இல்லை என்றால் எங்களிடம் தெரிவிக்கவும். நீங்கள் பங்கேற்க விரும்பினால் எங்களிடம் தெரிவிக்கவும். இதனால் நீங்கள் பிற்காலத்தில் மருத்துவமனியில் பெறும் சிகிச்சையை எந்த விதத்திலும் பாதிக்காது என்பதையும் நாங்கள் உங்களுக்கு தெரிவித்துக்கொள்ள விரும்புகிறோம்.

ஒப்புதல் கலந்துரையாடல் நடத்திய நபரின் கையொப்பம்

நான் _____ என்னும் குழந்தைக்குப் புரியும் மொழியில் இந்த ஆய்வினைப் பற்றி விவரித்துள்ளேன். குழந்தை இந்த ஆய்வில் பங்கு பெற ஒப்புக் கொண்டுள்ளது.

கலந்துரையாடல் செய்தவர் கையொப்பம் _____ தேதி

கலந்துரையாடல் செய்தவர் பெயர் _____

பாகம் 2–ஒப்புதல் சான்றிதழ்

நான் இந்த தகவலைப் படித்துத் தெரிந்து கொண்டேன் (படித்துத் தெரிவிக்கப்பட்டுள்ளேன்). எனது சந்தேகங்களைக் கேட்டு தெளிவு படுத்திக் கொண்டேன். பிற்காலத்திலும் எனது சந்தேகங்களைக் கேட்கலாம் என்பதையும் அறிந்து கொண்டேன்.

நான் இந்த ஆய்வில் பங்கு பெற விரும்புகிறேன்

(அல்லது)

எனக்கு இந்த ஆய்வில் பங்கேற்க விருப்பம் இல்லை, நான் கீழ் கண்ட ஒப்புதல் படிவத்தில் கையெழுத்திடவில்லை _____ (குழந்தையின் கையொப்பம்)

குழந்தை ஒப்புக்கொண்டால் மட்டும்

1. குழந்தையின் பெயர்
2. குழந்தையின் கையொப்பம்
3. தேதி

படிப்பறிவில்லாதவர்களாக இருந்தால்

ஒரு படித்த சாட்சி (குழந்தையின் பெற்றோரல்லாத, பங்கேற்பவரால் தேர்ந்தெடுக்கப்பட்ட ஒரு நபர்) கையொப்பமிடவேண்டும். படிப்பறிவில்லாதவர் தங்கள் கைநாட்டைப் பதிக்கவேண்டும். குழந்தையிடம் ஒப்புதல் பெறும் பொழுது இந்தப் படிவம் படிக்கப் பட்டதை நான் உடனிருந்து கவனித்தேன். பங்கேற்பாளர் தனது சந்தேகங்களைக் கேட்டு தெரிந்து கொள்ள வாய்ப்பளிக்கப்பட்டது என்பதை அறிந்து கொண்டேன். பங்கேற்பாளர் தனது ஒப்புதலை தனது சொந்த விருப்பத்தில் தான் தெரிவித்தார் என்று உறுதியளிக்கிறேன்.

சாட்சியின் பெயர் _____ பங்கேற்பவரின் கைநாட்டு

சாட்சியின் கையெழுத்து _____

தேதி _____

நான் பங்கேற்பாளருக்கு ஒப்புதல் படிவத்தை முழுவதும் படித்துக் காட்டினேன் / படித்துக் காட்டியதை கவனித்தேன். பங்கேற்பாளர் தனது சந்தேகங்களைக் கேட்டு தெரிந்து கொள்ள வாய்ப்பளிக்கப்பட்டது என்பதை அறிந்து கொண்டேன். பங்கேற்பாளர் தனது ஒப்புதலை தனது சொந்த விருப்பத்தில் தான் தெரிவித்தார் என்று உறுதியளிக்கிறேன்.

ஆய்வாளரின் பெயர் _____

பெற்றோர் ஒப்புதல் படிவம்

தலைப்பு:

குழந்தைகளுக்கு ஏற்படும் தீவிர டெங்குக் காய்சலுக்கும் இரத்தத்தில் உள்ள NS1 ஆன்டிஜனின் அளவுகளுக்கும் உள்ள சம்பந்தம் பற்றிய ஆய்வு

உங்கள் (மகன் / மகள் / குழந்தைகள்) இந்த ஆய்வுக்கு அழைக்கின்றேன். நான் குழந்தைகளுக்கு ஏற்படும் தீவிர டெங்குக் காய்சலுக்கும் இரத்தத்தில் உள்ள NS1 ஆன்டிஜனின் அளவுகளுக்கும் உள்ள சம்பந்தம் பற்றி கோயம்புத்தூரில் ஆய்வு நடத்த உள்ளேன்.

என் பெயர் **மரு. லாவண்யா .போ,** பூ சா கோ மருத்துவக் கல்லூரியில் குழந்தைகள் நலப் பிரிவில் ஜீனியர் ரெசிடென்டாக பணிபுரிகிறேன். இந்த ஆய்வு எனது படிப்பின் முழுமையான பூர்த்திக்கு அவசியமானதாகும்.

நான் இந்த ஆய்வில் உங்கள் (மகன் / மகள் / குழந்தைகள்) சேர்க்க அனுமதி கேட்கிறேன், ஏனெனில் நான் இந்த ஆய்வில் குழந்தைகள் பங்கேற்பார்கள் என்று எதிர்பார்க்கிறேன்.

நீங்கள் அனுமதுயளித்தால், நான் அல்லது பயிற்சி பெற்ற நபர்கள் ஒரு கேள்விப்படிவம் அளிப்போம்.

உங்கள் குழந்தைகளைப் பற்றி இந்த ஆய்வில் கண்டறியும் முடிவுகள் மிகவும் நம்பிக்கக்குறியதாக வைக்கப்படும். உங்கள் அனுமதியின் பெயரில் மட்டுமே வெளியிடப்படும். உங்கள் குழந்தையின் பதில்களோ அல்லது உங்கள் குழந்தையின் பெயரோ எந்த எழுத்து வடிவமாகவோ அல்லது வாய்வழி படிவமாகவோ வெளியிடப்படாது.

உங்கள் அனுமதியால், உங்களுக்கோ உங்கள் பிள்ளைகளுக்கோ பூ சா கோ மருத்துவமனையுடனான உறவில் எந்த பாதிப்பும் ஏற்படாது. உங்களுக்கு ஆய்வில் ஏதேனும் கேள்விகள் இருந்தால் என்னை கேளுங்கள். உங்களுக்கு பிற்காலத்தில் ஏதேனும் ஐயங்கள் இருந்தால் இந்த எண்ணை அழைக்கவும் (8012017880). உங்கள் குழந்தையின் பங்கேற்பில் ஏதேனும் சந்தேகம் உன்டெனில் இந்த எண்ணிற்கு (0422-2570170, Extn. 5818) அழைக்கவும்

இந்த ஒப்புதல் படிவத்தில் ஒரு பிரதியை நீங்கள் பெற்றுக்கொள்ளலாம். நீங்கள் இந்த ஆய்வில் பங்கேற்க உங்கள் (மகன் / மகள் / குழந்தைகள்) முடிவுசெய்கிறீர்களானால் கீழே கையெழுத்திட்டு அதற்கான ஒப்புதலை அளிக்க வேண்டும். உங்கள் கையெழுத்தின் பொருள் யாதெனில் நீங்கள் இந்த படிவத்தில் உள்ள தகவல்கள் அனைத்தையும் முழுமையாக படித்து உங்கள் (மகன் / மகள் / குழந்தைகள்) இதில் பங்கேற்க அனுமதி வழங்குகிறீர்கள் என அர்த்தம்.

பிற்காலத்தில் இந்த ஆய்வில்லிருந்து உங்கள் (மகன் / மகள் / குழந்தைகள்) பங்கேற்க வேண்டாம் என்று என்னினால் எனக்கு அறிவிக்கவும். உங்கள் விருப்பமின்மையை எந்நெரம் வேண்டுமென்றாலும் தெரிவிக்கவும். இது உங்கள் வருங்கால சிக்ச்சைமுறையை எந்த விதத்திலும் பாதிக்காது.

பெயர் (மகன் / மகள் / குழந்தைகள்):

பெற்றோர் அல்லது சட்டப்பூர்வமான பாதுகாவலரின் கையொப்பம் / தேதி

ஆய்வாளரின் கையொப்பம் / தேதி

PROFORMA

Name

Age

Sex

Weight

Height

Address

Fever Yes/No

Duration

Retroorbital pain Yes/No

Arthralgia Yes/No

Head ache Yes/No

Rashes Yes/No

Myalgia Yes/No

VITALS

- Temp BP mm/hg Heart rate /min Resp rate / min
- Urine output ml/kg/hour Capillary refilling time
- Pulse volume
- Peripheries warm/ cold
- Head ache yes/no
- Rashes yes/no
- Myalgia yes/no

VITALS

- Temp BP mm/hg Heart rate /min Resp
- Urine output ml/kg/hour Capillary refilling time
- Pulse volume
- Peripheries warm/ cold

INVESTIGATIONS:

- Platelet count-
- Haematocrit-
- Haemoglobin-
- Total count-
- NS1 Antigen Yes/No

Titre value

Day of illness

- Dengue serology

IgM Yes/ No Titre

IgG Yes/No Titre

MASTER CHART

SL.NO.	AGE	NS1 TITRE	DIAGNOSIS	DAY OF ILLNESS	ICU ADMN.	SEROLOGY
1	3	>11	Severe	4	Yes	secondary
2	9	<9	Mild	1		primary
3	5	9 – 11	Moderate	5		secondary
4	1	>11	Mild	4		secondary
5	7	<9	Moderate	2		primary
6	8	9 – 11	Mild	5		secondary
7	3	9 – 11	Severe	3	Yes	primary
8	17	>11	Mild	1		primary
9	6	<9	Moderate	3		secondary
10	3	<9	Moderate	5		primary
11	12	9 – 11	Mild	2		secondary
12	15	>11	Severe	4	Yes	primary
13	4	9 – 11	Mild	5		secondary
14	2	9 – 11	Moderate	2		primary
15	13	>11	Mild	1		primary
16	17	<9	Moderate	3		secondary
17	3	9 – 11	Mild	5		primary
18	13	9 – 11	Severe	3	Yes	secondary
19	2	<9	Moderate	4		primary
20	7	9 – 11	Mild	2		secondary
21	2	>11	Moderate	1		secondary
22	1	9 – 11	Moderate	4		primary
23	9	<9	Mild	5		secondary
24	16	>11	Mild	1		primary
25	13	(	Moderate	3		primary
26	2	<9	Mild	2		primary
27	1	>11	Severe	4	Yes	secondary
28	6	<9	Mild	1		primary
29	7	>11	Moderate	3		secondary
30	17	>11	Moderate	2		secondary
31	4	>11	Mild	4		secondary
32	2	<9	Moderate	5		primary
33	7	<9	Mild	1		primary
34	8	9 – 11	Moderate	2		secondary
35	2	9 – 11	Mild	5		primary
36	13	<9	Moderate	4		secondary
37	14	>11	Severe	3	Yes	primary
38	17	9 – 11	Moderate	1		secondary
39	6	9 – 11	Mild	3		primary
40	9	<9	Moderate	4		primary
41	1	9 – 11	Moderate	5		secondary
42	3	<9	Mild	2		primary
43	2	<9	Mild	3		secondary
44	6	9 – 11	Moderate	2		secondary
45	17	>11	Moderate	2		primary
46	12	<9	Mild	1		secondary
47	5	9 – 11	Moderate	4		primary
48	2	<9	Severe	3	Yes	secondary
49	14	>11	Moderate	1		secondary
50	16	>11	Mild	5		primary
51	17	<9	Moderate	2		primary
52	3	9 – 11	Moderate	5		secondary
53	2	9 – 11	Mild	1		secondary
54	5	<9	Mild	4		primary
55	1	>11	Moderate	1		secondary
56	13	>11	Severe	3	Yes	secondary
57	3	>11	Moderate	1		secondary
58	1	<9	Mild	3		primary
59	7	>11	Mild	4		secondary
60	5	>11	Moderate	5		primary
61	8	<9	Mild	1		secondary
62	9	9 – 11	Moderate	3		primary

SL.NO.	AGE	NS1 TITRE	DIAGNOSIS	DAY OF ILLNESS	ICU ADMN.	SEROLOGY
63	1	<9	Severe	4	Yes	secondary
64	17	<9	Mild	1		primary
65	3	9 – 11	Mild	2		secondary
66	7	>11	Moderate	4		secondary
67	4	>11	Mild	5		primary
68	2	9 – 11	Moderate	1		secondary
69	1	9 – 11	Mild	3		primary
70	8	>11	Severe	4	Yes	secondary
71	14	9 – 11	Mild	1		secondary
72	9	<9	Moderate	2		secondary
73	14	>11	Mild	3		primary
74	6	<9	Mild	5		primary
75	7	>11	Moderate	3		secondary
76	9	9 – 11	Mild	2		primary
77	17	>11	Moderate	4	Yes	primary
78	2	9 – 11	Mild	1		primary
79	5	>11	Moderate	5		primary
80	6	<9	Moderate	2		secondary
81	2	<9	Mild	4		primary
82	14	>11	Moderate	3		secondary
83	6	9 – 11	Mild	2		secondary
84	16	<9	Mild	4		primary
85	3	>11	Moderate	1		secondary
86	4	>11	Mild	5		secondary
87	2	9 – 11	Mild	4		secondary
88	5	>11	Severe	3	Yes	primary
89	17	9 – 11	Mild	1		primary
90	13	<9	Moderate	4		primary
91	4	>11	Mild	5		secondary
92	5	>11	Mild	2		primary
93	1	<9	Moderate	3		secondary
94	9	>11	Moderate	2		secondary
95	8	>11	Moderate	4		primary
96	3	>11	Mild	5		primary
97	5	9 – 11	Mild	1		secondary
98	1	>11	Severe	4	Yes	secondary
99	15	9 – 11	Moderate	5		secondary
100	13	>11	Moderate	2		primary
101	12	>11	Mild	4		primary
102	4	<9	Mild	3		secondary
103	8	<9	Moderate	5		primary
104	9	9 – 11	Mild	2		primary
105	3	<9	Moderate	1		secondary
106	2	>11	Mild	7		primary
107	13	>11	Moderate	2		secondary
108	4	<9	Severe	4	Yes	secondary
109	1	>11	Mild	1		primary
110	3	9 – 11	Mild	2		secondary
111	5	9 – 11	Moderate	5		secondary
112	2	<9	Mild	3		secondary
113	6	>11	Moderate	2		primary
114	7	<9	Mild	5		primary
115	9	<9	Moderate	1		secondary
116	1	9 – 11	Mild	2		primary
117	15	>11	Mild	4		secondary
118	2	<9	Severe	3	Yes	primary
119	17	>11	Mild	1		secondary
120	1	9 – 11	Mild	2		primary
121	1	>11	Moderate	4		primary
122	15	>11	Moderate	5		secondary
123	2	>11	Moderate	3		primary
124	13	9 – 11	Mild	1		secondary
125	5	<9	Moderate	4		primary

SL.NO.	AGE	NS1 TITRE	DIAGNOSIS	DAY OF ILLNESS	ICU ADMN.	SEROLOGY
126	7	>11	Severe	3	Yes	primary
127	9	<9	Moderate	4		primary
128	4	9 – 11	Moderate	1		secondary
129	2	>11	Mild	2		primary
130	1	9 – 11	Mild	5		secondary
131	2	>11	Moderate	2		primary
132	15	<9	Moderate	4		secondary
133	6	9 – 11	Mild	1		primary
134	2	9 – 11	Mild	5		secondary
135	5	<9	Moderate	2		secondary
136	8	>11	Moderate	3		primary
137	16	>11	Severe	4	Yes	primary
138	9	9 – 11	Moderate	3		secondary
139	12	<9	Moderate	1		secondary
140	3	>11	Mild	2		primary
141	9	<9	Moderate	4		secondary
142	1	<9	Mild	5		primary
143	3	>11	Mild	2		secondary
144	12	<9	Moderate	3		primary
145	5	<9	Moderate	1		secondary
146	7	>11	Mild	3		primary
147	3	>11	Severe	4	Yes	secondary
148	8	>11	Mild	1		primary
149	9	<9	Moderate	2		secondary
150	12	>11	Mild	5		secondary
151	3	9 – 11	Moderate	3		secondary
152	4	9 – 11	Moderate	1		secondary
153	13	9 – 11	Moderate	5		primary
154	4	<9	Mild	2		primary
155	13	>11	Moderate	4		primary
156	4	9 – 11	Mild	3		secondary
157	6	>11	Severe	4	Yes	primary
158	2	>11	Mild	1		secondary
159	12	>11	Moderate	3		primary
160	4	>11	Moderate	4		secondary
161	15	<9	Mild	5		primary
162	17	<9	Mild	2		secondary
163	2	<9	Moderate	3		secondary
164	4	<9	Mild	1		primary
165	7	9 – 11	Severe	4	Yes	primary
166	8	9 – 11	Mild	1		secondary
167	1	>11	Mild	2		primary
168	9	<9	Mild	4		primary
169	2	9 – 11	Moderate	2		secondary
170	8	9 – 11	Mild	3		primary
171	4	<9	Mild	1		primary
172	6	<9	Moderate	5		secondary
173	13	9 – 11	Mild	3		secondary
174	17	<9	Moderate	2		primary
175	2	9 – 11	Mild	1		secondary
176	4	>11	Mild	5		secondary
177	3	>11	Mild	2		secondary
178	12	>11	Severe	4	Yes	secondary
179	13	9 – 11	Mild	1		primary
180	17	9 – 11	Moderate	4		primary
181	1	<9	Mild	1		secondary
182	9	>11	Mild	3		secondary
183	4	>11	Moderate	4		primary
184	2	>11	Mild	5		secondary
185	9	9 – 11	Mild	1		primary
186	1	>11	Mild	2		secondary
187	3	<9	Mild	5		primary
188	7	<9	Severe	3	Yes	primary

SL.NO.	AGE	NS1 TITRE	DIAGNOSIS	DAY OF ILLNESS	ICU ADMN.	SEROLOGY
189	9	9 – 11	Mild	2		primary
190	3	>11	Mild	1		primary
191	1	>11	Moderate	3		secondary
192	2	9 – 11	Mild	5		secondary
193	6	<9	Mild	1		primary
194	8	9 – 11	Moderate	2		secondary
195	2	<9	Mild	4		secondary
196	8	>11	Mild	3		secondary
197	9	>11	Mild	1		primary
198	2	<9	Severe	4	Yes	primary
199	4	<9	Mild	1		secondary
200	2	>11	Moderate	2		secondary
201	8	9 – 11	Moderate	5		primary
202	9	9 – 11	Mild	3		primary
203	2	<9	Mild	1		secondary
204	4	>11	Moderate	3		primary
205	1	>11	Mild	5		secondary
206	8	>11	Moderate	2		primary
207	4	<9	Mild	1		secondary
208	7	9 – 11	Mild	3		primary
209	9	9 – 11	Severe	4	Yes	primary
210	2	>11	Mild	1		primary
211	1	>11	Mild	2		secondary
212	14	<9	Mild	4		secondary
213	2	<9	Moderate	2		secondary
214	8	9 – 11	Moderate	1		primary
215	6	>11	Moderate	3		secondary
216	2	<9	Mild	4		primary
217	3	<9	Mild	1		secondary
218	4	>11	Moderate	3		secondary
219	7	9 – 11	Mild	5		primary
220	1	<9	Mild	2		primary
221	7	9 – 11	Mild	3		secondary
222	3	>11	Severe	4	Yes	primary
223	6	>11	Severe	3	Yes	secondary
224	1	9 – 11	Mild	1		secondary
225	9	>11	Moderate	2		primary
226	17	<9	Mild	5		secondary
227	2	<9	Mild	3		primary
228	4	>11	Mild	2		primary
229	15	9 – 11	Moderate	1		primary
230	2	<9	Moderate	4		secondary
231	6	<9	Mild	3		primary
232	13	>11	Mild	1		secondary
233	2	<9	Moderate	5		secondary
234	12	9 – 11	Mild	2		primary
235	15	>11	Mild	4		primary
236	2	9 – 11	Mild	3		secondary
237	6	<9	Moderate	1		primary
238	7	9 – 11	Mild	3		secondary
239	9	>11	Mild	2		primary
240	8	9 – 11	Moderate	4		primary
241	1	<9	Mild	1		secondary
242	13	9 – 11	Moderate	5		secondary
243	2	<9	Mild	3		primary
244	1	>11	Mild	2		secondary
245	5	<9	Moderate	1		primary
246	7	>11	Mild	4		secondary
247	13	>11	Moderate	1		secondary
248	4	<9	Mild	3		secondary
249	17	9 – 11	Mild	4		primary
250	12	>11	Moderate	2		secondary