"A STUDY OF CLINICO-HAEMATOLOGICAL PROFILE OF PATIENTS WITH DENGUE FEVER"

By

DR. VARUN DESAI

Dissertation submitted to the

SBKS MEDICAL INSTITUTE & RESEARCH CENTRE

SUMANDEEP VIDYAPEETH, PIPARIA, VADODARA

In partial fulfillment

Of the requirements for the degree of

DOCTOR OF MEDICINE

IN

MEDICINE

Under the Guidance of

DR. (COL) V. P. SINGH

(PROFESSOR OF THE DEPARTMENT OF MEDICINE)

SBKS MEDICAL INSTITUTE & RESEARCH CENTRE,

PIPARIA, VADODARA

YEAR 2015-2018

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SUMANDEEP VIDYAPEETH
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Date: 21/12/16
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Ref: Your study synopsis entitled "Study of clinico hematological profile with dengue fever" Submitted to the SV IEC for approval.

Sub: Approval for conducting the referenced study

Dear Dr. Varun,

The Sumandeep Vidyapeeth Institutional Ethics Committee (SV IEC) is in receipt of your above mentioned study document and as the research study classifies in the minimal risk category; as recommended by HRRP SBKS MI&RC. The SV IEC approves your study to be conducted in the presented form.

The approval remains valid for a period of 1 year. In case the study is not initiated within one year, the Ethics Committee expects to be informed about the reason for the same and a fresh approval will have to be obtained subsequently.

The Sumandeep Vidyapeeth Institutional Ethics Committee expects to be informed about the progress of the study (every 6 months), any Serious Adverse Event (SAE) occurring in the course of the study, and if any changes are made in the protocol or patient information/informed consent the SVIEC needs to be informed about this in advance and an additional permission is required to be taken. The SV IEC also requires you to submit a copy of the final study report.

22/12/2016

Dr. Niraj Pandit
Member Secretary
SV Institutional Ethics committee

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Date: 25th September 2017

STUDY COMPLETION CERTIFICATE

This is to certify that your study entitled: "Study of Clinico Hematological Profile with Dengue Fever" Research Project was done by **"Dr. Varun Desai"** (PG Student, Dept of Medicine, S.B.K.S MI & RC, Dhiraj Hospital, Piparia, Waghodia road, Vadodara-391760, Gujarat) and it was conducted to the satisfaction of the Sumandeep Vidyapeeth Institutional Ethics committee.

Dr. Niraj Pandit
Member Secretary
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SUMANDEEP VIDYAPEETH

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"A STUDY OF CLINICO-HAEMATOLOGICAL PROFILE OF PATIENTS WITH DENGUE FEVER" AT DHIRAJ GENERAL HOSPITAL, PIPARIA** is a bonafide and genuine research work carried out by me under the guidance of **DR.(COL) V. P. SINGH, PROFESSOR OF DEPARTMENT OF MEDICINE, SBKS Medical Institute & Research Centre, Piparia, Vadodara.**

Date:
Place:PIPARIA

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DR VARUN DESAI

SUMANDEEP VIDYAPEETH

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **"A STUDY OF CLINICO-HAEMATOLOGICAL PROFILE OF PATIENTS WITH DENGUE FEVER" AT DHIRAJ GENERAL HOSPITAL, PIPARIA** is a bonafide research work done by **DR. VARUN DESAI** under my guidance and in partial fulfillment of the requirement for the degree of **M.D. Medicine.**

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ENDORSEMENT BY THE HOD & DEAN

OF THE INSTITUTION

This is to certify that the dissertation entitled **"A STUDY OF CLINICO-HAEMATOLOGICAL PROFILE OF PATIENTS WITH DENGUE FEVER" AT DHIRAJ GENERAL HOSPITAL, PIPARIA** is a bonafide research work done by **DR. VARUN DESAI** under the guidance of **DR.(COL) V. P. SINGH, professor of Department of Medicine.**

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DR. HETAL PANDYA
Professor of Medicine

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Place:PIPARIA

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ACKNOWLEDGEMENT

I am very much indebted and wish to express my earnest thanks to my guide ***Dr. (col) V. P. Singh****, M.D Professor Department of Medicine, Dhiraj Hospital, Sumandeep Vidyapeeth, Piparia Village, Vadodara district for his meticulous guidance, expert advice, valuable suggestions, continuous supervision and constant encouragement that motivated me throughout the course of study.*

I am extremely grateful to my HOD and Professor ***Dr.Hetal Pandya****, M.D for her thought provoking guidance and encouragement in preparing this dissertation.*

I also thank Dr. Mansukh Shah, Chancellor, Sumandeep Vidyapeeth; Dr. Dixit Shah, Vice Chancellor, Sumandeep Vidyapeeth; Dr. G.V. Shah, Dean, SBKSMI & RC, Sumandeep Vidyapeeth; Dr (COL) Rakesh Kumar Sareen, Medical Superintendent, Dhiraj Hospital, Sumandeep Vidyapeeth; M.M.Sattigeri, Registrar, Sumandeep Vidyapeeth and Dr. Seth, Research Director, Sumandeep Vidyapeeth for their help, co-operation and permission to carry out my studies in this institution.

I express my gratitude to all colleagues and friends for their wholehearted help in carrying out this study. I would like to thank my family, my parents, ***Dr Kanji B Rabari and Dr Ila K Rabari*** *and my brother* ***Rohan Desai*** *for their constant encouragement and guidance and always helping me to never lose focus in my studies and for being with me in all the steps of my life. My heartfelt gratitude to all my patients who submitted themselves most gracefully and whole heartedly participated in this study. To my colleagues, life during post-graduation would not have been the same without you.*

And I would like to thank GOD for giving me energy, inspiration and courage to complete this task.

Finally, my wife, my strength, my hope, my everything, ***Dr Payal Desai****, thank you for being by my side through it all and giving me all the much needed moral support, dedication and patience.*

DR. VARUN DESAI

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ABSTRACT

INTRODUCTION

Dengue fever is an infection that has been prevalent in India for more than 2 centuries. The number of reported cases of dengue are on the rise globally and South-east Asia region has a large population which is at risk and susceptible to dengue. Dengue epidemics have a significant economic and health toll. Epidemic dengue fever (DF) and dengue hemorrhagic fever (DHF) have emerged as a global public health problem in recent decades. In fact, the problem has become hyperendemic in many urban, peri-urban and rural areas, with frequent epidemics. The South-East Asia Region is one of the regions at highest risk of DF/DHF. Dengue has shown a wide clinico haematological profile from symptomatically mild disease to extreme fatal disease with multi organ dysfunctions Clinically.

AIMS AND OBJECTIVES

The aim and objective of the studies are to study the various clinical and haematological manifestations of patients with Dengue fever, to understand better the risk factors that lead to Dengue Hemorrhagic fever and Dengue Shock Syndrome and also to understand the various haematological and biochemical investigation findings in cases with dengue fever

METHODS

Written informed consent was taken from each patient and study was explained to each patient. Patients were selected based on inclusion/exclusion criteria. Patients of Dengue fever were enrolled from OPD patients or patients admitted to the wards/ICU.

Appropriate history was taken and clinical examination of these patients was carried out. Routine investigations like Hb, CBC, RFT's, LFT's, RBS, Serum electrolytes were carried out for each patient from the Central Laboratory. Radiological investigations like Chest X-ray, Ultrasound of the abdomen or thorax, were carried out as per the routine standard followed, based on presentation of the patient. There was no extra cost for these investigations incurred by the patient for being in the study. Invasive investigations like Pleural fluid tapping and Analysis of the fluids were carried out where-ever necessary. Written informed consent of the patient was taken prior to any such investigation. All the data accumulated was compiled properly and conclusions were drawn from the same.

RESULTS

There is high incidence of dengue fever than dengue hemorrhagic fever or dengue shock syndrome. Males are slight more commonly affected than females in the ratio 1.27:1. Fever associated with myalgia, headache and retro-orbital pain was the most common presentation present in all the patients in our study. Rash and positive tourniquet test were the most common bleeding manifestations (34%) followed by haematuria (19%) and epistaxis (13%). NS1 antigen was the most common to be positive in 31 patients (62%), IgM and IgG were positive in 11 and 1 patient respectively Mean platelet counts were 94000 per cu ml, with lowest count was 10000 per cubic ml. Among all the patients in the study, 56% had normal total wbc counts, with 10% had leucocytosis and 34% had leukopenia Mean haematocrit was 38.19 ±1.92 %, increased levels were found in 20 of patients (40%). Acute kidney injury (S. Creatinine ≥1.5) was present in 24% of the study patients. Hypokalaemia was present in 16 % of the patients. Abnormal SGPT was found in 28% of total patients in

this study, with 100% in dengue shock syndrome and 42.9% in dengue hemorrhagic fever patients. SGOT was found to be raised in 31 patients with 100% in dengue hemorrhagic and shock syndrome patients. Direct hyperbilirubinemia was found in 6% of patients, with 0% in patients with dengue fever while 75% in patients with dengue shock syndrome. APTT was raised in 28% of total patients with being 75% of dengue shock syndrome patients. Total bilirubin was raised in 7 patients in our study (14%), 1 patient in dengue fever group , 3 patients with dengue hemorrhagic fever.

Ascites was present in 4 patients (8%) which were all patients with dengue shock syndrome. Pleural Effusion was present all of DSS patients with 6 patients with dengue hemorrhagic fever patients and overall in 10 patients (20%). Hepatomegaly and splenomegaly was present in 18 patients (36%) in our study , being all the patients of dengue hemorrhagic fever and dengue shock syndrome patients, while 7 patients (17.9%) with dengue fever patients.

CONCLUSION

In summary, young adults are at risk of dengue the most. Diagnosis of this vector borne disease is usually straight forward and no advanced clinical skill required, this disease has variety of clinical and haematological presentation, with early diagnosis and treatment we can reduce the burden of this disease especially in the endemic countries like India. Further studies will be needed to keep track of the changing epidemiological and clinical trends of dengue. More extensive studies to assess DHF are needed. Also a look into the differences of primary and secondary dengue and their profile is essential.

INTRODUCTION

Dengue fever is an infection that has been prevalent in India for more than two centuries. The first recorded epidemic of clinically dengue like illness occurred at Chennai in 1780 and the dengue virus was isolated for the first time almost simultaneously in Japan and Calcutta in 1943–1944. The first proven epidemic of Dengue occurred along the East coast of India in 1963-64. The first full-blown epidemic of the severe form of the illness, the dengue hemorrhagic fever/ dengue shock syndrome occurred in North India in 1996.[1] *Aedes aegypti* is the vector for transmission of the disease. Vaccines or antiviral drugs are not available for dengue viruses; the only effective way to prevent epidemic dengue fever/dengue hemorrhagic fever (DF/DHF) is to control the mosquito vector, *Aedes aegypti* and prevent its bite.

Year	Place
1780	Madras
1824-25	Rangoon to Madras
1844-49	Kanpur, Calcutta
1852-56	Widespread
1870-73	Bombay, Calcutta, Madras
1897-99	Bombay
1901-07	Madras
1907-13	Calcutta, Pune, Meerut
1920-26	Lucknow, Bombay, Calcutta

1927-28	Coimbatore
1930-33	Madras
1934-36	Madras
1940-45	Calcutta

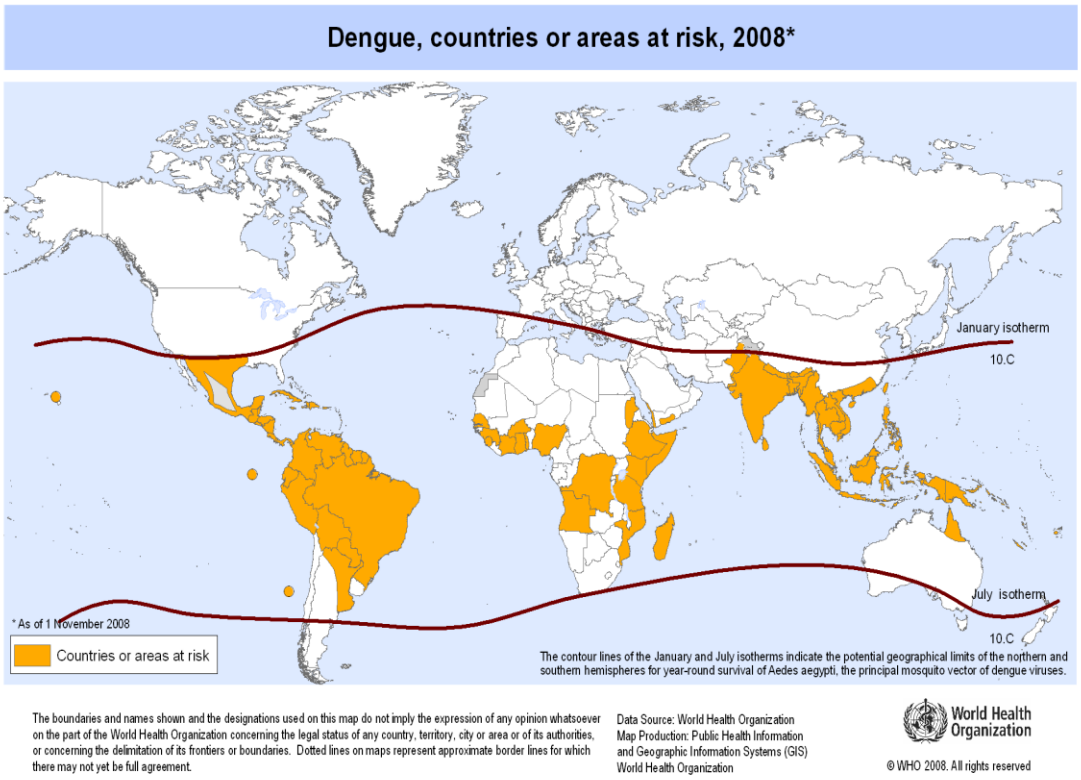
(Adapted from Chaturvedi U C and Nagar R 2008 Dengue and dengue hemorrhagic fever: Indian perspective; *J. Biosci* 2008.33:429–41)

The number of reported cases of dengue are on the rise globally and South-east Asia region has a large population which is at risk and susceptible to dengue. Worldwide, an estimated 2.5 billion people are at risk of infection; approximately 975 million of who live in urban areas in tropical and sub-tropical countries in Southeast Asia, the Pacific and the Americas.[2] Dengue epidemics has a significant economic and health toll. In endemic countries in Asia and the Americas, the burden of dengue is approximately 1,300 disability-adjusted life years (DALYs) per million populations, which is similar to the burden of tuberculosis, in these regions.[3]

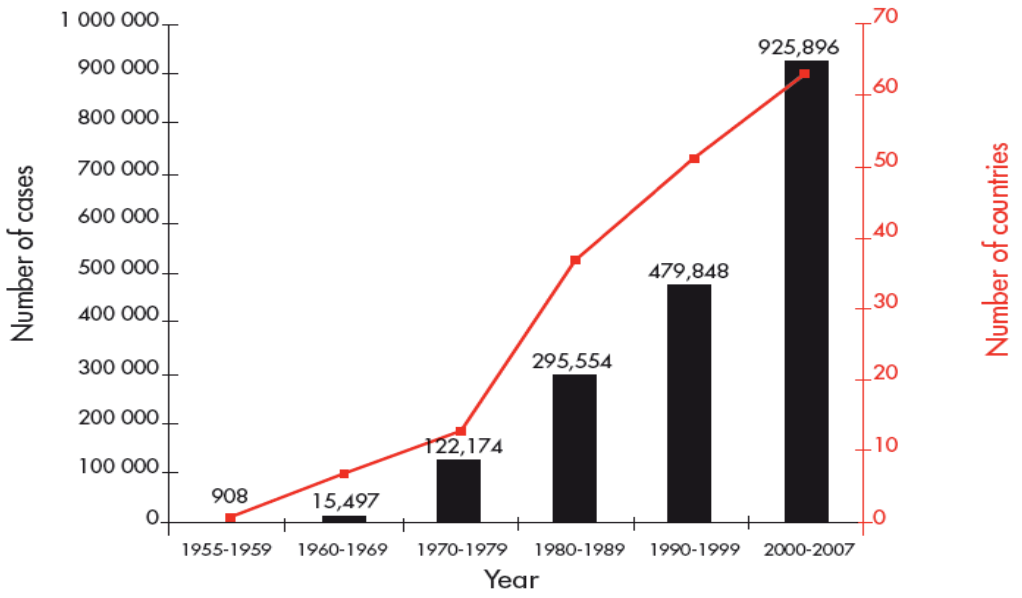
Epidemic dengue fever (DF) and dengue hemorrhagic fever (DHF) have emerged as a global public health problem in recent decades. In fact, the problem has become hyper endemic in many urban, peri-urban and rural areas, with frequent epidemics. The South-East Asia Region is one of the regions at highest risk of DF/DHF, accounting for 52% of the global risk. Dengue outbreaks now occur in India, as in other high-burden countries in the Region, such as Indonesia, Myanmar and Thailand.[4] With over 16000 cases reported in India every year, which forms only the tip of the iceberg, a study into the various aspects of dengue fever is imperative.[5]

The Need for the Study-

At present, data on adult dengue infections in South Asia is limited and hence there is need for studies to better understand the laboratory and clinical features of dengue infections. Hence we take up this study to profile patients of dengue fever at a tertiary care Dhiraj Hospital.



Areas at increased risk of dengue[4]



Rising trend in number of cases of dengue reported since 1955 to 2007[4]

AIM

- To study the various clinical and haematological manifestations of patients with Dengue fever

OBJECTIVES

- To understand better the risk factors that lead to Dengue Hemorrhagic fever and Dengue Shock Syndrome
- To understand the various haematological and biochemical investigation findings in cases with dengue fever

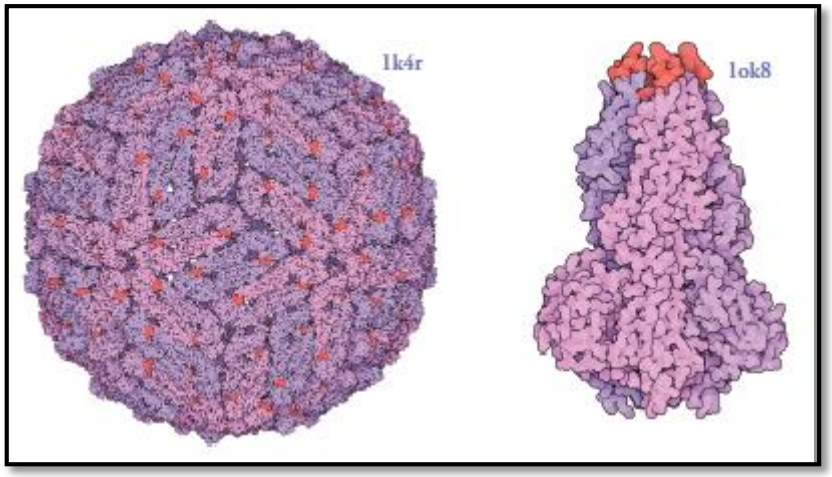
REVIEW OF LITERATURE

1) The Agent-

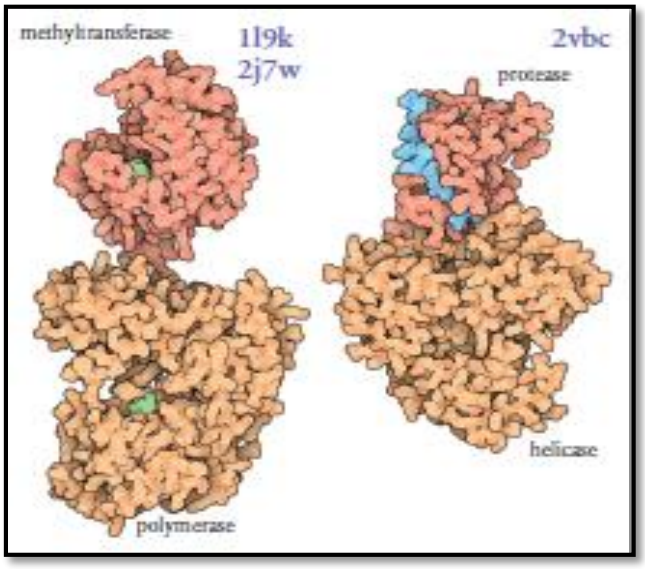
Dengue fever is caused by the Dengue virus (DENV), which is a single stranded RNA virus (11 kilo bases long) with an icosahedral nucleocapsid and covered by a lipid envelope. The virus is in the family Flaviviridiae, genus flavivirus and the type specific virus is yellow fever.[6]

The dengue virus has 4 related but antigenically distinct serotypes: DENV-1, DENV-2, DENV-3 and DENV-4. Genetic studies of sylvatic strains suggest that the 4 serotypes evolved from a common ancestor in primate populations approximately 1000 years ago and that all 4 emerged into a human urban transmission cycle 500 years ago in either Asia or Africa.[7] Albert Sabin speciated these viruses in 1944. Each serotype has a different genotype. Viral genotype and serotype, and the sequence of infection with different serotypes, appear to affect disease severity.

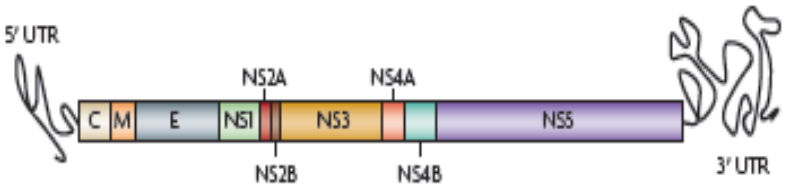
Its genome is about 11000 bases that codes for three structural proteins, capsid protein C, membrane protein M, envelope protein E; seven non-structural proteins, NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5; and short non-coding regions on both the 5' and 3' ends.[8, 9] Further classification of each serotype into genotypes often relates to the region where particular strains are commonly found or were first found.



Structure of Dengue virus- Envelope protein (left) and infectious form with smooth coat and icosahedral symmetry (right); the red tip binds to lysozomes and helps release RNA into the cytoplasm.[10,11]



Structure of Proteins- NS5 (left) with methyltransferase and polymerase activity and NS3 (right) with protease and helicase activity; the blue strand associated with NS3 is part of NS2B protein. All help in viral replication inside the cell.[12, 13, 14]



Dengue virus genome

There is approximately 65% homology between different serotypes of dengue virus. Despite these differences each of these serotypes result in identical illnesses in humans and have similar ecological niches.[22]

2) The Vector –

Dengue viruses are transmitted by the bite of an infected *Aedes* (subgenus *Stegomyia*) mosquito. Globally, *Aedes aegypti* is the predominant highly efficient mosquito vector for dengue infection, but the Asian tiger mosquito *Aedes albopictus*, and other *Aedes* species can also transmit dengue with varying degrees of efficiency. *Aedes* mosquito species have adapted well to human habitation, and are often seen breeding in small amounts of stagnant water. Humans are their preferred targets.[15]

Female *Aedes* are daytime feeders. They inflict an innocuous bite, usually on the back of the neck or ankles, and are easily disturbed during a blood meal, causing them to move on and finish a meal on another individual, making them efficient vectors. Not uncommonly, entire families develop infection within a 24-36 hour period, presumably from the bites of a single infected mosquito. *Aedes aegypti* is also known to be the vector for chikungunya virus and yellow fever virus. The mosquito can be identified by white markings on the legs and the mark in the form of a lyre on the thorax and is hence also known as the “tiger mosquito”.

Mosquitoes acquire the infection when they feed on the carrier of the virus. The mosquito can transmit dengue if it immediately bites another host. In addition, transmission occurs after 8-12 days of viral replication in the mosquito's salivary glands (extrinsic incubation period). The virus doesn't adversely affect the mosquito.[15] The mosquito remains infected for the remainder of its life. The life span of *Aedes aegypti* is 21 days (15-65). Eggs of the mosquito can withstand desiccation for prolonged periods. There are rare reports of dengue transmission vertically and also through needle stick injuries.[16,17]

3) The Host –

Humans serve as the primary reservoir for dengue. Persons with dengue virus in their blood can transmit the viruses to the mosquito 1 day before the onset of the febrile period. The patient remains infectious for the next 6-7 days. Once inoculated, the incubation period of the virus is 3-14 days while viral replication takes place in target dendritic cells. Infection of target cells of the reticuloendothelial system, such as dendritic cells, hepatocytes, and endothelial cells, result in production of immune mediators that help shape the initial and subsequent viral infections with response to them.[18,19,20]

4) Pathogenesis –

After inoculation into the skin after mosquito bite, dengue viruses infect immature dendritic cells through the non-specific receptor dendritic cell specific ICAM3-grabbing non-integrin (DC-SIGN).[21,22,23] Infected dendritic cells mature and migrate to local or regional lymph nodes where they present viral antigens to T cells, which initiates the cellular and humoral immune response. There is also evidence of abundant replication of DENVs in liver parenchymal cells and in macrophages in lymph nodes, liver and spleen, as well as in peripheral blood monocytes.[24] Both *in vitro* and *in vivo*, macrophages and monocytes participate in antibody dependent enhancement (ADE).[25,26,27] ADE occurs when mononuclear phagocytes are infected through their Fc receptors by immune complexes that form between DENVs and non-neutralizing antibodies. These nonneutralizing antibodies result from previous heterotypic dengue infections or from low concentrations of dengue antibodies of maternal origin in infant sera.[28] The cocirculation of four DENV serotypes in a given population might be augmented by the ADE phenomenon.[29] Various hypotheses for pathogenesis are [30] -

a) DENV tropism:

In vitro data and autopsy studies suggest that three organ systems play an important role in the pathogenesis of DHF/DSS: the immune system, the liver, and endothelial cell (EC) linings of blood vessels. During the feeding of mosquitoes on humans, DENV is presumably injected into the bloodstream, with spill over in the epidermis and dermis, resulting in infection of immature Langerhans cells (epidermal dendritic cells [DC]) and keratinocytes.[31,32] Infected cells then migrate from site of infection to lymph nodes, where monocytes and macrophages are recruited, which become targets

of infection. Consequently, infection is amplified and virus is disseminated through the lymphatic system. As a result of this primary viremia, several cells of the mononuclear lineage, including blood-derived monocytes[33], myeloid differentiating cells[34,35] and splenic and liver macrophages[36,37] are infected. DENV has also been shown to have tropism for circulating mononuclear cells in blood and for cells residing in the spleen, lymph nodes, and bone marrow of infected AG129 mice.[38]

A review of the literature describing findings on autopsy samples from a total of 160 fatal cases, mostly children or young adolescents (4 to 18 years old) who died within 36 hours of developing shock revealed, in order of frequency, the presence of DENV in cells in the skin, liver, spleen, lymph node, kidney, bone marrow, lung, thymus, and brain.[24,39,40,41]

In vitro studies have shown that all DENV serotypes can actively replicate in EC[42], and infection results in functional rather than morphological damage. It is not clear whether EC of different vascular-bed systems have different susceptibilities to DENV infection. Although increased peripheral microvascular permeability has been shown to occur in both DHF and DSS patients[43], it is conceivable that EC from the pulmonary and abdominal territories react in a specific way to either infection with or the response to DENV infection[44,45], resulting in the selective vascular leakage syndrome characteristic of DHF/ DSS.

Several studies suggest that vascular damage or dysfunction is central in the pathogenesis of DHF/DSS.[46,47,45] It is interesting to note that selective apoptosis of the microvascular EC in pulmonary and intestinal tissues has been detected in fatal cases of DHF/DSS, providing a possible explanation for the profound plasma leakage seen in pleural and peritoneal cavities. In this regard, it is worth mentioning that the major

nonstructural protein 1 (NS1) of DENV has been shown to bind preferentially to EC of lung and liver tissues.[48] It has been hypothesized that recognition of NS1 by anti-NS1 antibodies could then contribute to the selective pulmonary vascular leakage.

b) Virus virulence :

According to the virus virulence hypothesis, certain DENV strains are responsible for more severe disease. It has also been proposed that intraepidemic evolution of the circulating DENV might be responsible for increased severity of disease. Epidemiological observations in the Americas and in Singapore suggested that the sequence of infection with particular serotypes and the time interval between primary infection and secondary infection may play an important role in the development of DHF.

Epidemics with high incidences of DHF have been linked to primary infection with DENV-1 followed by infection with DENV-2 or DENV-3.[49,50,51] Furthermore, these studies indicated that the longer the interval between primary and secondary infections, the higher the risk of developing severe disease. In addition, age has been shown to influence the outcome of disease following a secondary infection with heterologous DENV. In Asia, the risk of severe disease is greater in children than in adults, in contrast to the Americas, where the adult population is mainly affected and infection results in milder disease. This difference in disease severity caused by Asian and American genotypes correlated with structural differences in the two strains of DENV.[52]

The observation that DHF/DSS is seen primarily in a relative small percentage of secondary DENV infections and to a much lesser extent in primary infections even with allegedly virulent strains suggests that host factors must be crucial determinants of severe disease development.

c) Activation of complement system :

With regard to DENV, investigators noticed that around the time of defervescence, when plasma leakage may become apparent, high levels of the activation products C3a and C5a are measured in the plasma, followed by an accelerated consumption and a marked reduction of the complement components in patients with DSS.[53,54] Therefore, it was hypothesized that complement activation plays an important role in the pathogenesis of dengue.

It has been proposed that NS1 is an important trigger for complement activation.[55] Binding of heterotypic antibodies to NS1 expressed on infected cells may result in complement activation. In addition, it is believed that NS1 released from infected cells can directly activate complement factors present in the fluid phase.[55]

Several groups have shown that both IgG1 and IgG3 were the predominant subclasses involved in the specific antibody response in human DENV infections.[56,57] Both IgG subclasses can fix and activate the complement system effectively, whereas IgG2 and IgG4 are less effective in this respect.

d) Transient autoimmunity :

Antibodies produced during a DENV infection have been shown to cross-react with some self-antigens, but it is not clear if production of these antibodies is associated with secondary DENV infections. For instance, antibodies recognizing a linear

epitope in the E protein have been shown to bind human plasminogen and inhibit plasmin activity.[58] The presence of serum antibodies specific to NS1 also has been shown to correlate with disease severity.[59] Cross reaction of anti-NS1 with cells of the liver, EC, and platelets could be at the basis of this observation.

e) Host genetic factors :

Differences in disease severity can be seen at both the individual and population levels. Several epidemiological studies indicated that genetic factors constitute important components in disease susceptibility. Several human HLA class I and II alleles are associated with development of DHF.

List of HLA and non-HLA genetic factors associated with pathogenesis of DHF/DSS:

i. Vitamin D receptor polymorphism[60]

ii. FCγRIIa receptor polymorphism[60]

iii. G6PD[61]

iv. MBL2[62]

v. TGF-β[63]

vi. TNF-α308A polymorphism[64]

vii. CTLA-4[63]

viii. Transporters associated with antigen presentation and human platelet antigen[65]

ix. DC-SIGN polymorphism[66]

x. HLA class I alleles A*01, A*0207, A*24, B*07, B*46, B*51[67]

xi. HLA class II alleles DQ*1, DR*1, DR*4[67]

f) Antibody dependent enhancement (ADE) :

In most acute virus infection models, the presence of antibodies, both neutralizing and nonneutralizing, correlates with control, elimination, and eventually protection. However, a possible detrimental role of virus-specific antibodies has been described for several viruses as measured by in vitro enhancement of infection of cells[68,69], a phenomenon that is not restricted to viral pathogens only. This in vitro phenomenon was also described for DENV infection, and epidemiological studies have shown an increased risk of developing DHF/DSS after a secondary DENV infection.[70]

These observations led to the conclusion that subsequent infection of preimmune individuals with a different DENV serotype could exacerbate rather than mitigate disease, a phenomenon that was claimed to be caused by antibodies and termed antibody-dependent enhancement (ADE) of disease.[71] Several subsequent epidemiological studies provided further circumstantial evidence for the role of preimmunity in the pathogenesis of DHF. ADE could result in infection of a higher number of target cells, which could lead to the high viral load observed in many studies.[72] Despite several clinical studies, evidence for the role of ADE in human disease, such as in DENV infections, remains circumstantial.

An alternative or complementary hypothesis is that FcγR mediated entry suppresses the antiviral immune response. For instance, a study with Ross River virus showed that viral entry via the FcγR pathway could suppress antiviral genes and enhance IL-10 production in murine macrophages, while entry via the normal cellular receptor did not change the antiviral environment.[73,74] Furthermore, it was shown that virus replication was necessary in order to promote IL-10 expression. Unfortunately, the Fc receptor that was involved in ADE was not identified. It was also shown that DENV

infection of THP-1 cells via FcR suppressed the transcription and production of IL-12, IFN-γ, TNF-α, and NO but enhanced expression of the anti-inflammatory cytokines IL-6 and IL-10, indicating that ADE of DENV infection also resulted in a milieu that promoted viral replication. These results must be interpreted with caution, however, since the effect of ADE of infection on gene expression may be cell dependent.

The role of preimmunity conferred by vaccination against DENV or other flaviviruses in the pathogenesis of DENV infections has been studied in limited numbers of subjects. One study conducted in Thailand reported no differences in the incidence of DHF in children who had received a live-attenuated DENV vaccine and unvaccinated controls at 6 to 8 years after vaccination.[75]

g) Cross reactive T-cell response :

Although memory T cells cross-reactive with a heterologous virus can provide partial protective immunity, they can also cause substantial immunopathology. The role of $CD8^+$ T cells during DENV infection is not entirely clear, but they may play a role in clearing infection as well as in immunopathogenesis.[76,77] It is worth noting that a consistent finding in all examples of T-cell-mediated pathology during acute or persistent viral infections is morphological tissue damage as a result of cytolysis or inflammation induced by the high numbers of effector T cells. The efficiency of activated T cells in clearing virus-infected cells is dependent on the avidity of the T-cell receptor (TCR) for the HLA-peptide complex, and it is assumed that cross-reactive T cells of low avidity for heterologous virus are not protective.

During the acute phase of a secondary infection of humans with heterologous DENV, highly cross-reactive $CD8^+$ T cells with high avidity for the infecting virus are preferentially activated.[78,79] The majority of these cross-reactive T cells produce high concentrations of pro- and anti-inflammatory cytokines such as IFN-γ, TNF-α, and IL-13 but somewhat lower levels of IL-10. These high-avidity cross-reactive $CD8^+$ T cells die through apoptosis, but it is not clear whether cells die as a result of activation-induced cell death or whether apoptosis is selectively induced by cross-reactive epitopes. Other studies have also suggested that epitopes can regulate the level of proinflammatory cytokines produced by T cells. Alternatively, low-avidity cross-reactive $CD8^+$ T cells would be preferentially expanded.[81] These crossreactive T cells react differently to the heterologous epitopes than to homologous epitopes by producing high levels of proinflammatory cytokines, but they lose their cytolytic activity. Delayed virus clearance would prolong activation of such cross reactive $CD8^+$ T cells, which then results in the production of high levels of cytokines such as TNF-α, IL-6, or other soluble factors that affect vascular permeability. The phenomenon where cross-reactive memory T cells for the primary infecting virus are more efficiently activated, due to the increased frequency and higher activation state of memory cells, has been called original antigenic sin (OAS). This phenomenon has also been described for LCMV in mice.[80,81] During a secondary infection with a heterologous serotype, cross-reactive epitopes preferentially reactivate the larger number of memory T cells against the priming virus more effectively than they activate naïve T cells. However, in accordance to what has been described for several other systems, it is possible that during a heterologous DENV infection, only a very small subset of cross-reactive memory T cells will be stimulated to expand because of a narrowing TCR repertoire. This narrowing of the TCR repertoire in combination

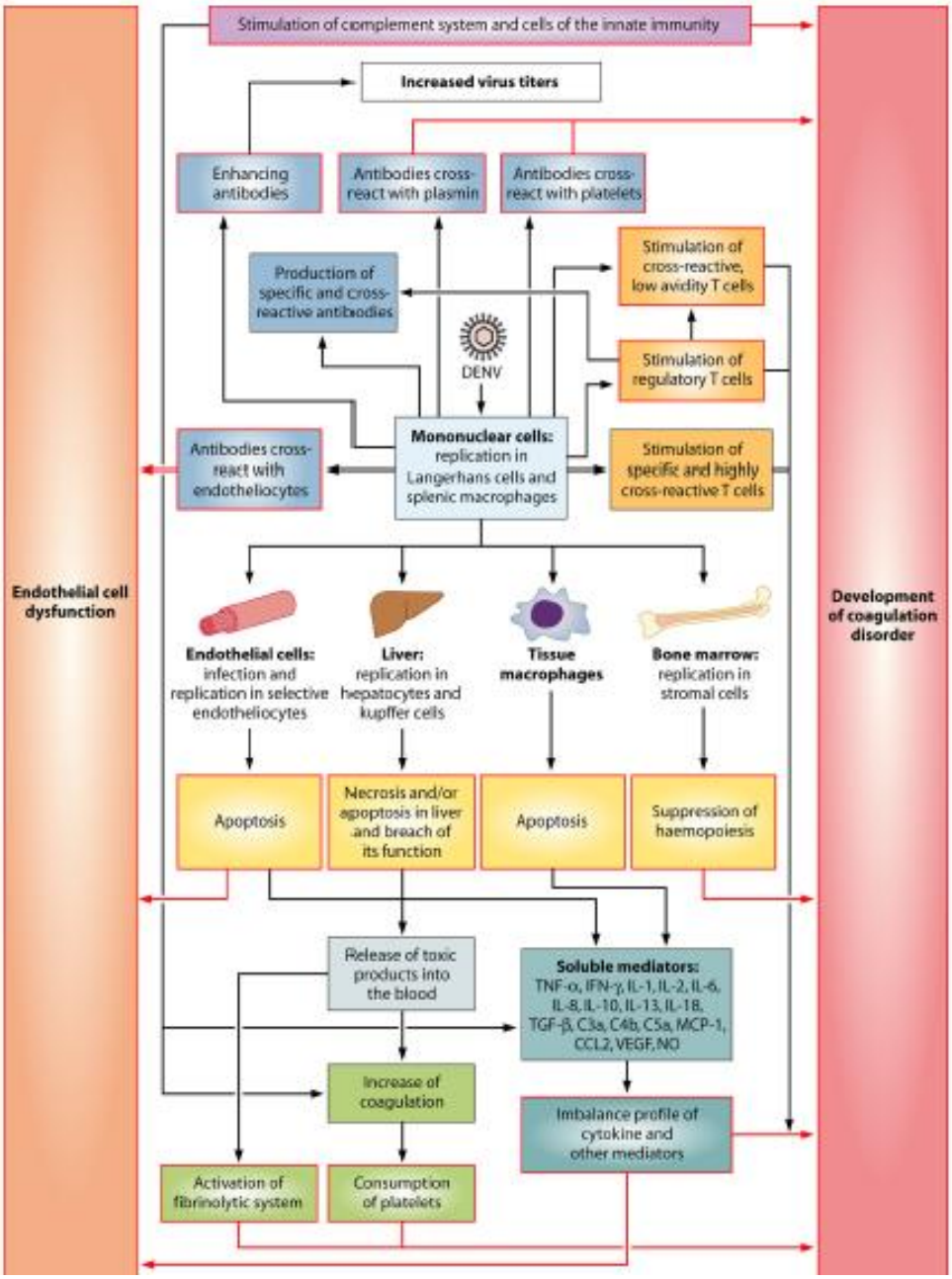
with the fact that each individual has a unique TCR specificity (private TCR[82]) would result in dominant responses that are unique for individuals. This could explain the variability seen in disease outcome upon secondary infection with heterologous DENV.

h) Soluble Factors :

One of the most daunting challenges in DENV research is the identification of soluble factors that can mediate, either alone or in combination the functional changes induced in EC that are associated with the increased plasma leakage. Several studies have shown that concentrations of multiple cytokines and other mediators, as well as soluble receptors, are significantly increased during severe dengue infections. Higher plasma levels of IL-1β, IL-2, IL-4, IL-6, IL-7, IL-8, IL-10, IL-13, IL-18, TGF-1β, TNF-α, and IFN-γ have been found in patients with severe DENV infections, in particular in patients with DSS[83,84,85]. These studies analyzed samples from infants, children, and adults infected with different DENV serotypes. It is reasonable to assume that synergistic interactions between these cytokines will occur. Other mediators and soluble factors found to be increased in severe disease include vascular endothelial growth factor (VEGF), granulocyte-macrophage colony-stimulating factor, monocyte chemoattractant protein 1 (MCP-1), macrophage migration inhibitory factor, thrombopoietin, soluble vascular cell adhesion molecule 1 (VCAM-1), soluble ICAM-1, von Willebrand factor antigen, thrombomodulin, E-selectin, tissue factor (TF), plasminogen activator inhibitor 1 (PAI-1), and tissue plasminogen activator [46,86,87,88,89].

$CD4^+$ T cells have been shown to produce a unique cytokine called the cytotoxic factor, with peak amounts measured in DHF/DSS cases[90,91]. The validity of these observations has to be confirmed by independent experiments. Clearly, there is a substantial redundancy between cytokines (i.e., the lack of one specific cytokine may be compensated for by another cytokine with overlapping activities), making it difficult to explain DHF/DSS pathogenesis on the basis of a single cytokine. It is more likely that multiple cytokines contribute simultaneously in a complex way to the development of DHF/ DSS.

THE INTEGRATED VIEW OF PATHOGENESIS:



Adapted from Martina B.E, Koraka P, Osterhaus A.D. Dengue virus pathogenesis: an integrated view. *Clin Microbiol Rev.* 2009 Oct; 22:564-81.

5) Clinical Features –

Dengue is seen as one common monsoon related illness in India but is endemic in many areas. Most patients experience a prodrome of chills, erythematous mottling of the skin, and facial flushing (a sensitive and specific indicator of dengue fever). The prodrome may last for 2-3 days. Children younger than 15 years usually have a nonspecific febrile syndrome, which may be accompanied by a maculopapular rash. Classic dengue fever begins with sudden onset of fever, chills, and severe (termed breakbone) aching of the head, back, and extremities, as well as other symptoms. The fever lasts 2-7 days and may reach 41°C. Fever that lasts longer than 10 days is probably not due to dengue.[92]

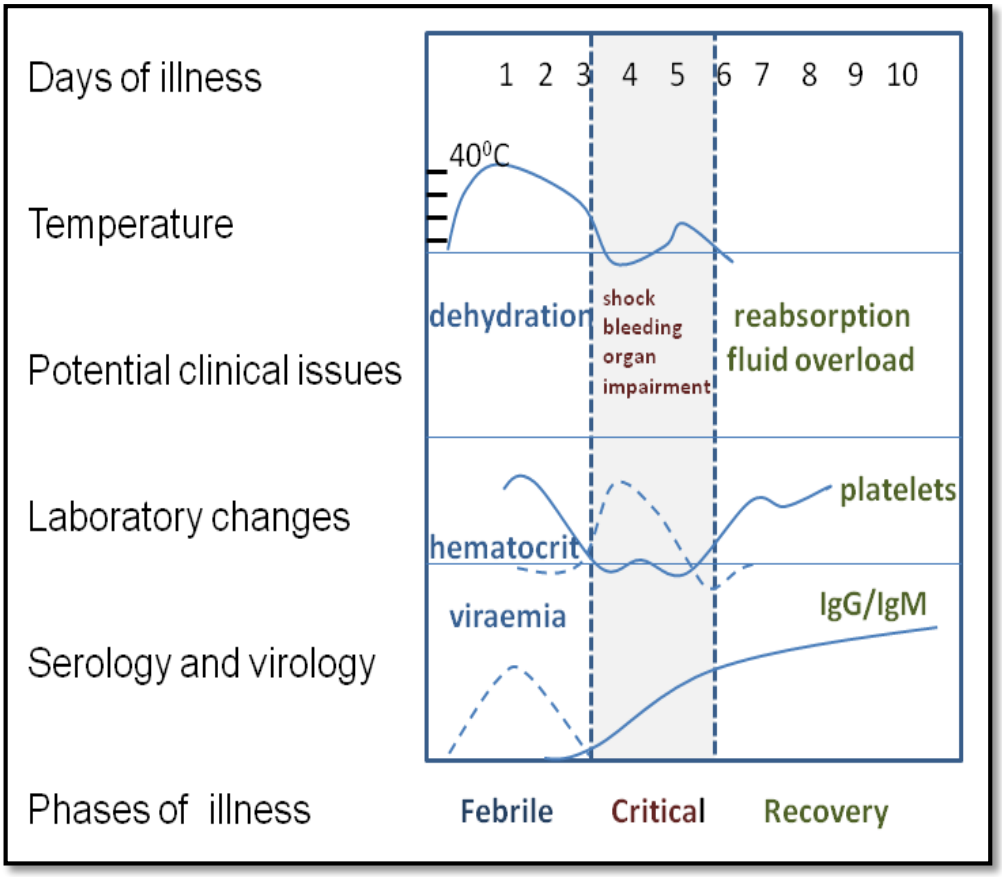
Typically, people infected with dengue virus are asymptomatic (80%) or only have mild symptoms such as an uncomplicated fever. Others have more severe illness (5%), and in a small proportion it is life-threatening. The incubation period (time between exposure and onset of symptoms) ranges from 3–14 days, but most often it is 4–7 days.[93,94,95]

The course of infection is divided into three phases: febrile, critical, and recovery. The febrile phase involves high fever, potentially over 40 °C (104 °F), and is associated with generalized pain and a headache; this usually lasts two to seven days. Nausea and vomiting may also occur. A rash occurs in 50–80% of those with symptoms in the first or second day of symptoms as flushed skin, or later in the course of illness (days 4–7), as a measles-like rash. Some petechiae can appear at this point, as may some mild bleeding from the mucous membranes of the mouth and nose. The fever itself is classically biphasic in nature, breaking and then returning for one or two days,

although there is wide variation in how often this pattern actually happens. Hence the name is given "Saddle-back fever".[96,97,98]

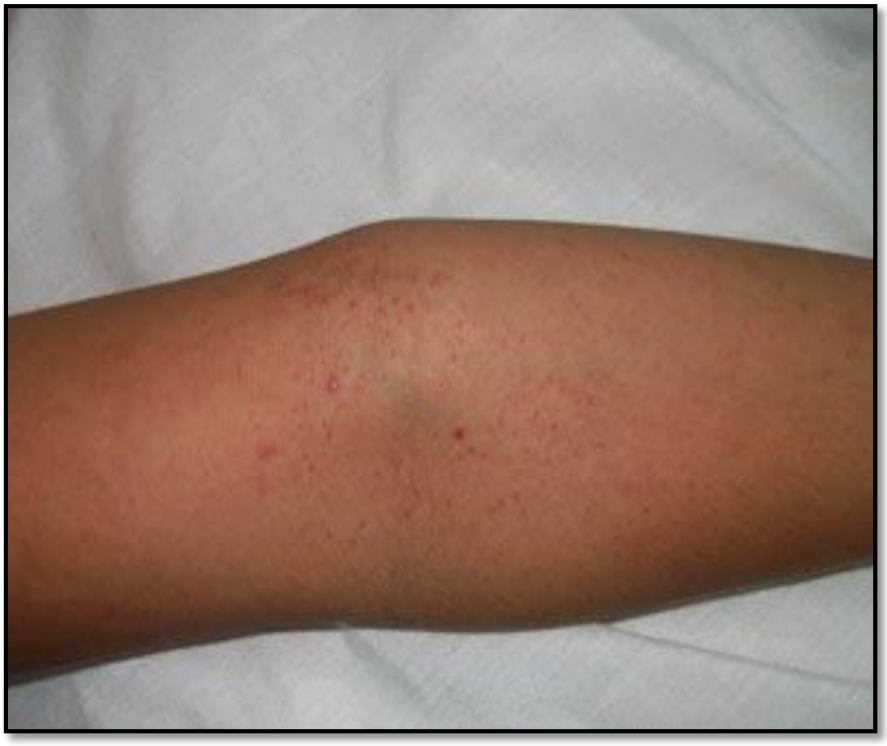
In some people, the disease proceeds to a critical phase around the time fever resolves and typically lasts one to two days. During this phase there may be significant fluid accumulation in the pleural and peritoneal cavities due to increased capillary permeability and leakage. This leads to hypovolemia and shock.[99] During this phase, organ dysfunction and severe bleeding, typically from the gastrointestinal tract, may occur.[100] Shock (dengue shock syndrome) and hemorrhage (dengue hemorrhagic fever) occur in less than 5% of all cases of dengue, however those who have previously been infected with other serotypes of dengue virus ("secondary infection") are at an increased risk.[101] This critical phase, while rare, occurs relatively more commonly in children and young adults.

The recovery phase occurs next, with resorption of the leaked fluid into the bloodstream. This usually lasts two to three days. The improvement is often striking, and can be accompanied with severe itching and a slow heart rate.[99,100] Another rash may occur with either a maculopapular or a vasculitic appearance, which is followed by peeling of the skin. There is risk of cerebral edema and seizures during this phase.[96]



Clinical course of Dengue fever[99]

Dengue Hemorrhagic Fever and Dengue shock syndrome: Typical cases of dengue hemorrhagic fever are characterised by four clinical manifestations- high fever, hemorrhagic manifestations and often, hepatomegaly and circulatory failure. Moderate to marked hepatomegaly with concurrent hemoconcentration is a distinctive laboratory finding of DHF. The major pathophysiological change in DHF which makes it severe and also differentiates it from DF is plasma leakage as manifested by hemoconcentration, serous effusions and hypoalbuminemia. The most common hemorrhagic phenomenon is a positive tourniquet test, easy bruising and bleeding from venepuncture sites.[102]



Tourniquet test- Inflate blood pressure cuff to midway between systolic and diastolic pressure for 5 mins. Positive if >20 petechiae per sq.inch[102]

DSS is characterised by the features of DHF with rapid weak pulse with narrowed pulse pressure or hypotension with cold clammy extremities with restlessness. If not appropriately treated at the earliest, the risk of death is very high. The patients remain conscious till the terminal stage and shock is short and profound, i.e. the patients die within 12-24 hours or recover rapidly with fluid replacement. Good prognostic signs of convalescence are good urine output and return of appetite.[102]

Case definitions [102]

Probable Case	Acute Febrile illness with two or more of the following Headache, retro-orbital pain, myalgia, arthalgia, rash, hemorrhagic manifestations, leucopenia and Supportive Serology OR occurrence at the same time and location as other confirmed cases of dengue fever
Confirmed case	A case confirmed by laboratory criteria i.e.: Isolation of dengue virus from serum or autopsy samples; or Demonstration of fourfold or greater change in reciprocal IgG or IgM antibody titres to one or more dengue virus antigens in paired serum samples; or Demonstration of dengue virus antigen in autopsy tissue, serum or cerebrospinal fluid samples by IHC, immunofluoroscence or ELISA; or Detection of dengue virus genomic sequences in autopsy tissue, serum or CSF by PCR

Clinical Case Definition for Dengue Hemorrhagic fever: Absolute criteria -

i. Fever, or recent history of acute fever

ii. Hemorrhagic manifestations

iii. Low platelet count (100,000/mm3 or less)

iv. Objective evidence of "leaky capillaries":

- elevated hematocrit (20% or more over baseline)
- low albumin
- pleural or other effusions

Clinical case definition of Dengue shock syndrome: 4 criteria of DHF +

i. evidence of circulatory failure manifested indirectly by all of the following

- Rapid and weak pulse
- Narrow pulse pressure (<20 mmHg) or hypotension for age
- Cold clammy extremities and altered mental status

ii. Frank shock is direct evidence of circulatory failure

DF/DHF	Grade	Symptoms	Laboratory findings
DF		Fever with 2 or more of following: Headache, Retro-orbital pain, Myalgias, Arthalgias	Leucopenia, occasionally thrombocytopenia. No e/o plasma loss
DHF	I	Above plus positive tourniquet test	Platelets<100000/mm^3, Hct rise >=20%
DHF	II	Above sign plus spontaneous bleeding	Platelets<100000/mm^3, Hct rise >=20%
DHF*	III	Above sign plus circulatory failure (weak pulse, hypotension, restlessness)	Platelets<100000/mm^3, Hct rise >=20%
DHF*	IV	Above signs with undetectable BP and pulse	Platelets<100000/mm^3, Hct rise >=20%

WHO classification of dengue fever[102] (DHF III & IV are categorised as DSS)

Dengue fever is commonly seen in young adults. Children are less affected as compared to adults but atypical manifestations are seen more often in children.[103] Males are more commonly affected as compared to females with the ratio being approximately 2:1.[104] Also mortality is more among males with DHF being more common amongst male patients.

Age wise distribution of patients (Tank Arun G et al[104])

Age group	Year 2009	Year 2010	Year 2011
<1	2	0	0
1-4	8	3	0
5-14	95	46	3
15-44	266	199	10
>45	22	17	0
Total	393	265	13

Fever is the most common clinical manifestation followed by headache.[105] Bleeding manifestations are common in the form of petechial rash. Bleeding manifestations are more common in DHF than in DF.[106] Vomiting and abdominal pain are other common manifestations seen in Dengue. Atypical manifestations are plenty as mentioned below.

Clinical features of patients (Singh NP et al[107])

Clinical features	No.(%) of cases (n=185)
Fever	185 (100)
Headache	114 (61.6)
Backache	107 (57.8)
Vomiting	94 (50.8)
Abdominal pain	39 (21)
Hepatomegaly	20 (10.8)
Splenomegaly	10 (5.4)
Icterus	8 (4.3)
Ascites	2 (1.08)
Pleural effusion	2 (1.08)
Loss of consciousness	3 (1.6)

Atypical manifestations of Dengue fever (S Gulati et al[108])

Neurological	**Encephalopathy** **Encephalitis/aseptic meningitis** **Intracranial hemorrhage/thrombosis** **Mononeuropathies/polyneuropathies** **GBS** **Myelitis**
Gastrointestinal/Hepatic	Hepatitis/ fulminant hepatic failure Acalculous cholecystitis Acute pancreatitis Febrile diarrhea Acute parotitis
Renal	Hemolytic uremic syndrome Renal failure

Cardiac	Myocarditis Conduction abnormalities Pericarditis
Respiratory	ARDS Pulmonary hemorrhage
Musculoskeletal	Myositis Rhabdomyolysis
Lymphoreticular	Spontaneous splenic rupture Lymph node infarction

6) Laboratory features and Diagnosis –

Characteristic findings in dengue fever are thrombocytopenia (platelet count < 100 x 10^9/L), leukopenia, and mild-to-moderate elevation of aspartate aminotransferase and alanine aminotransferase values. Jaundice and acute liver failure are uncommon. Peak liver enzyme levels occur later than other complications in adults studied prospectively in Vietnam. Enzyme levels begin to rise during the early stage and peak during the second week. Clinically severe involvement was found to be idiosyncratic and infrequent but did contribute to severe bleeding.[109,110]

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

In dengue hemorrhagic fever, thrombocytopenia was more prolonged and the number of atypical lymphocytes was higher, while the other haematological abnormalities presented daily evolution similar to those in classic dengue.[111] A systematic review found that patients with dengue had significantly lower total WBC, neutrophil, and

platelet counts than patients with other febrile illnesses in dengue-endemic populations.[112] A study from China by Lin SF et al. suggested that leukopenia in dengue fever may be caused by virus-induced destruction or inhibition of myeloid progenitor cells. Thrombocytopenia may result from by destruction of peripheral platelet or bone marrow megakaryocytes by viruses which consequently reduce the platelet production.[113]

Metabolic panel in dengue fever shows increase in transaminase levels in most patients with almost 44 % of patients having transaminitis in a study from Brazil. The average AST and ALT levels in this study were 93.3 U/l and 86.6 U/l. Also elevations and progression to acute hepatitis was more common in females, in DHF and in secondary infections.[114]

Raised serum BUN levels are commonly seen in patients with dengue who present with dehydration and also in Dengue shock syndrome. Acute kidney injury with raised serum creatinine can occur in upto 13 % patients with Dengue fever. AKI in dengue infections is associated with neurological involvement, prolongation of aPTT, greater length of hospital stay and increased mortality.[115] Also Dengue shock syndrome was found to be an independent risk factor for AKI in a study from Taiwan.[116] AKI in dengue was more commonly associated with hypotension with need for inotropes, coexistent hepatitis, sepsis, MODS and lower albumin levels in a study from south India by Mehra N et al.[117]

Hypoalbuminemia is commonly associated in dengue fever with plasma leakage. It is one of the defining criteria for DHF. It is seen in upto one third of the patients of dengue fever. As it is commonly associated with DHF, it is an independent risk factor for mortality in dengue fever.[106] There is a case report of dengue leading to reversible

proteinuria, the pathogenesis of which is not elucidated.[118] Coagulation studies may help guide management of patients with severe bleeding manifestations. Dengue can lead to acute hepatitis which manifests with a raised INR. Also aPTT can be prolonged with dengue fever with low fibrinogen and high fibrin degradation products as markers of DIC. Thrombin time may be prolonged and also a reduction in α-antiplasmin may be seen in cases with severe dengue fever.[102]

The most common electrolyte abnormality associated with dengue fever is hyponatremia with upto 40% showing mildly low sodium levels in study by R Joshi et al 2011.[119,120] This is commonly associated with disturbances in sensorium. Also it is seen in cases of dengue encephalopathy with no signs seen on MR imaging. Also commonly associated with dengue is hypokalemia seen in upto 28 percent of dengue cases and rarely manifesting as hypokalemic paralysis. The mechanism for hypokalemia is not yet known.[121]

Comparison of Laboratory parameters between DF and DHF (Karoli R et al 2012[106])

	Total Dengue positive (n=138)	Dengue fever (n=96)	Dengue hemorrhagic fever (n=42)	P value
Thrombocytopenia (platelets <100000)	123 (89%)	81 (84%)	42 (100%)	0.52
Abnormal PT/aPTT	47 (34%)	13 (14%)	34 (81%)	0.01*
Elevation of transaminases	127 (92%)	87 (90%)	40 (95%)	0.87
Hypoproteinemia (<5.5 g/dL)	47 (34%)	12 (13%)	35 (83%)	0.01*

Serum amylase elevations can be seen occasionally in cases of Dengue hemorrhagic fever. Also bulky and enlarged pancreas were seen in upto 29% patients with DHF in a study from Indonesia.[122] Pancreatitis is a rare complication of dengue fever with few case reports in literature.[123] Hence a high index of suspicion is needed in patients to diagnose pancreatitis associated with dengue.

Ultrasonography is a potentially timely, cost-effective, and easily used modality in the evaluation of potential dengue hemorrhagic fever. Positive and reliable ultrasonographic findings include fluid in the chest and abdominal cavities, pericardial effusion, and a thickened gallbladder wall. Thickening of the gallbladder wall may presage clinically significant vascular permeability.[124] The utility of previous studies was limited because patients underwent only a single scan. However, in a study by Srikiatkhachorn et al, daily serial ultrasonographic examinations of the thorax and abdomen proved useful in the evaluation of patients with suspected dengue hemorrhagic fever.[124] Plasma leakage was detected in some patients within 3 days of fever onset. Pleural effusion was the most common sign. Based on ultrasonographic findings, dengue hemorrhagic fever was predicted in 12 patients before haemoconcentration criteria had been met. Acalculous cholecystitis is a common finding on ultrasound seen in upto 40% patients in a study from Eastern India.[125] Hepatomegaly and splenomegaly are also features commonly seen on ultrasound with upto 23% patients having liver enlargement and 9% having splenic enlargement in a study by Sharma et al from 1998.[126]

Rare manifestations of dengue in the form of myositis and myocarditis are also seen with raised CPK levels.[127] Patients may present with weakness of muscles and also there is a case report by Wali et al[128] of reversible cardiac dysfunction associated with

dengue seen on 2D ECHO by initial depressed ejection fraction which improved spontaneously. Also ECG may show conduction disturbances in the form of sinus bradycardia or bundle branch blocks.

Dengue in pregnancy has not been studied extensively but it is clear that various manifestations of dengue are masked by pregnancy hence diagnosis is a challenge.[129] Also laboratory manifestations of dengue are unclear with pregnancy as total counts are higher during pregnancy which masks the leucopenia in dengue and also the haemoconcentration of DHF is masked by hemodilution of pregnancy. Also the fetal passage of antibodies is initially protective for the child but later puts the child at greater risk of DHF/DSS. Also there is a significant risk of hemorrhage and shock. Perrett et al[130] reported that serious dengue usually occurred in patients who acquired their infection at or near term. Sharma et al[131] reported increased incidence of neural tube defects when dengue was associated with pregnancy but other febrile illnesses can also cause the same, hence the association is not clear. There are case reports of vertical transmission of dengue with a review done by Sirinavin et al.[132] No data is available on early pregnancy losses due to dengue.

SERODIAGNOSIS OF DENGUE:

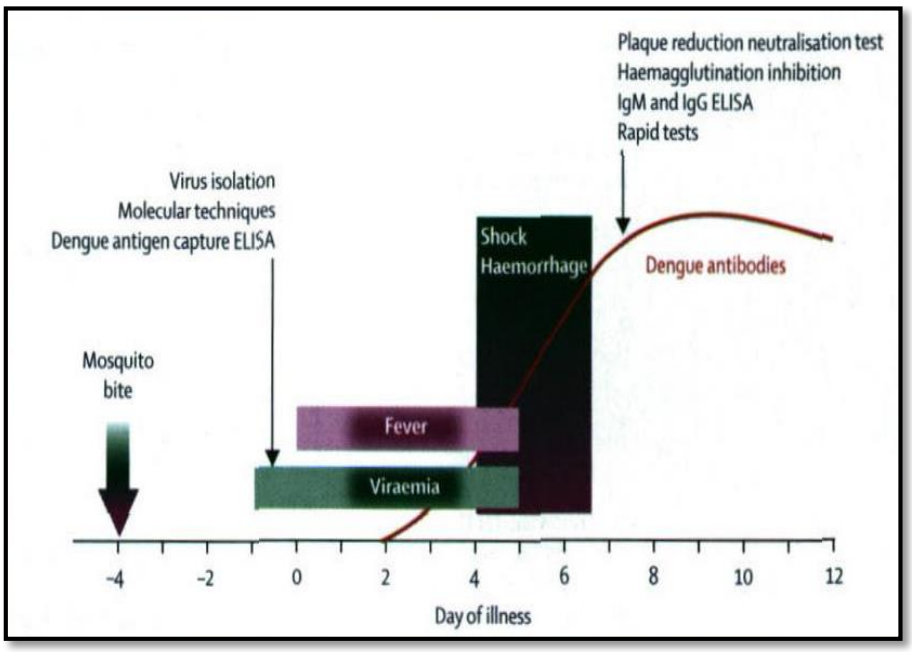
Serodiagnosis of dengue infection is very important to confirm diagnosis in patients with high index of suspicion. Multiple modalities for detection are present. Laboratory diagnosis methods for confirming dengue virus infection may involve detection of the virus, viral nucleic acid, antigens or antibodies, or a combination of these techniques. After the onset of illness, the virus can be detected in serum, plasma, circulating blood cells and other tissues for 4–5 days. During the early stages of the disease, virus isolation, nucleic acid or antigen detection can be used to diagnose the

infection. At the end of the acute phase of infection, serology is the method of choice for diagnosis.

Antibody response to infection differs according to the immune status of the host. IgM antibodies are the first immunoglobulin isotype to appear. These antibodies are detectable in 50% of patients by days 3-5 after onset of illness, increasing to 80% by day 5 and 99% by day 10. IgM levels peak about two weeks after the onset of symptoms and then decline generally to undetectable levels over 2–3 months. Anti-dengue serum IgG is generally detectable at low titres at the end of the first week of illness, increasing slowly thereafter, with serum IgG still detectable after several months, and probably even for life.[133]

During a secondary dengue infection (a dengue infection in a host that has previously been infected by a dengue virus, or sometimes after non-dengue flavivirus vaccination or infection), antibody titres rise rapidly and react broadly against many flaviviruses. The dominant immunoglobulin isotype is IgG which is detectable at high levels, even in the acute phase, and persists for periods lasting from 10 months to life. Early convalescent stage IgM levels are significantly lower in secondary infections than in primary ones and may be undetectable in some cases, depending on the test used. To distinguish primary and secondary dengue infections, IgM/IgG antibody ratios are now more commonly used than the haemagglutination-inhibition test (HI).

Course of dengue infection and timing of diagnosis (Halstead SB 2007[134])



Interpretation of dengue diagnostic tests (adapted from Dengue and Control study (DENCO study)[135])

Highly suggestive	Confirmed
1. IgM + in a single serum sample 2. IgG + in a single serum sample with a HI titre of 1280 or greater	One of the following: 1. PCR + 2. Virus Culture + 3. IgM seroconversion in paired sera 4. IgG sero-conversion in paired sera or fourfold IgG titre increase in paired sera

Advantages and disadvantages of tests used to diagnose dengue[133,136]

Diagnostic tests	Advantages	Limitations
Nucleic acid detection	• Most sensitive and specific • Possible to identify serotype • Early appearance (preantibody)-so opportunity to impact patient management	• Potential false positive due to contamination • Expensive • Needs expertise and expensive laboratory equipment • Not possible to differentiate primary and secondary infection
Isolation in cell culture and identification using immunofluorescence	• Specific • Possible to identify serotype by using specific antibodies	• Needs expertise and facility • Takes more than 1 week • Not possible to differentiate primary and secondary infection
Antigen detection	• Easy to perform • Opportunity for early diagnosis	• Not as sensitive as virus isolation or RNA detection
Serologic tests: IgM tests Seroconversion: 4 fold rise in HI or ELISA IgG titres between acute and convalescent sample	• Useful for confirmation of acute infection • Least expensive • Easy to perform • Can distinguish primary and secondary infection	• May miss cases as IgM levels may be low or undetectable in some secondary infections • Needs two samples • Delay in confirming diagnosis

To differentiate between primary and secondary infections, the methods used are IgM/IgG ratio and hemagglutination inhibition (HI) test. If patient's IgM/IgG ratio is less than 1.2 (using patient's sera at 1:100 dilution) or 1.4 (using patient's sera at 1:20 dilution) is suggestive of a secondary infection.[136]

The haemagglutination-inhibition (HI) test is based on the ability of dengue antigens to agglutinate red blood cells (RBC) of ganders or trypsinized human O RBC. Anti-dengue antibodies in sera can inhibit this agglutination and the potency of this inhibition is measured in an HI test. Optimally the HI test requires paired sera obtained upon hospital admission (acute) and discharge (convalescent) or paired sera with an interval of more than seven days. The assay does not discriminate between infections by closely related flaviviruses (e.g. between dengue virus and Japanese encephalitis virus or West Nile virus) nor between immunoglobulin isotypes. The response to a primary infection is characterized by the low level of antibodies in the acute-phase serum drawn before day 5 and a slow elevation of HI antibody titres thereafter. During secondary dengue infections HI antibody titres rise rapidly, usually exceeding 1:1280. Values below this are generally observed in convalescent sera from patients with primary responses.

7) Management of Dengue fever:

Patients can be grouped into 3 categories for treatment after initial assessment:[134]

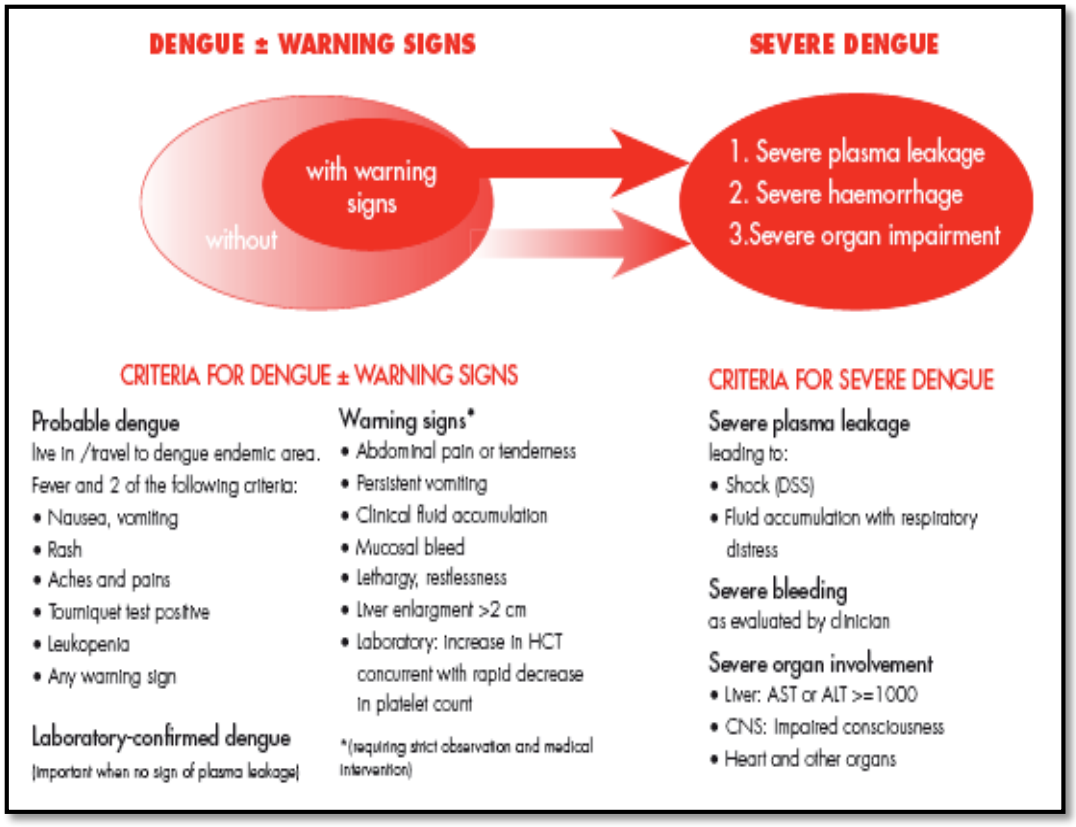
Group A are those who can be managed on a domiciliary basis. Patients are advised generous intake of fluids, oral rehydration solutions. Patients are advised to watch for any signs of deterioration and advised to follow up if they notice the same. These patients are prescribed with paracetamol but other NSAIDs are avoided due to their platelet inhibition.

Group B is patients who are admitted for inpatient management. These are patients who have warning signs at the time of admission, or have an underlying illness which complicates management of dengue like diabetes mellitus, pregnancy, chronic haemolytic disease, etc. For these patients after initial assessment of hematocrit, start intravenous normal saline or Hartmann's solution at 5-7 mL/kg/hr for 1-2 hours, after which slow it to 3-5 mL/kg/hr for 3-4 hours. Reassess hematocrit and monitor urine output to maintain it at 0.5-1 mL/kg/hr. If vital signs are worsening and hematocrit rises further, increase rate of infusion to 10-12 mL/kg/hr and look for signs of plasma leakage and organ failure. Referral to higher centre for expert management is to done under the following circumstances[134]:

- early presentation with shock (on days 2 or 3 of illness);
- severe plasma leakage and/or shock;
- undetectable pulse and blood pressure;
- severe bleeding;
- fluid overload;
- organ impairment (such as hepatic damage, cardiomyopathy, encephalopathy,
- encephalitis and other unusual complications.

Group C are patients with severe dengue and require emergency management for the same. These patients have severe plasma leakage leading to shock, severe hemorrhage or severe organ impairment in the form of renal failure, hepatic failure, encephalopathy, cardiomyopathy or ARDS. These patients are to be shifted to a facility with intensive care facilities and a functioning blood bank. These patients need early and judicious replacement of intravenous fluids and also monitoring of

blood pressure. Hematocrit should be monitored to see if there is further hemoconcentration, fluid resuscitation should be intensified. If there is drop in hematocrit consideration to blood transfusion should be given.



Suggested dengue classification and levels of severity[135]

The goals of fluid resuscitation include improving central and peripheral circulation (decreasing tachycardia, improving blood pressure, pulse volume, warm and pink extremities, and capillary refill time <2 seconds) and improving end-organ perfusion – i.e. stable conscious level (more alert or less restless), urine output ≥ 0.5 ml/kg/hour, decreasing metabolic acidosis. Appropriate treatment for complications like mechanical ventilation for ARDS, plasma support for acute liver failure and hemodialysis for acute renal failure should be instituted.

The preferable new treatment for dengue would be an antiviral drug.[136] At present, a specific antiviral drug is not available; however, there have been a lot of attempts to discover one. In phytomedicine, several sulphated polysaccharides extracted from seaweeds have been studied and high antiviral activity against dengue virus has been observed[137]. In modern medicine, ribavirin, glycyrrhizin and 6-azauridine are reported to have cytostatic and inhibitory effects on the dengue virus[138]. An adenosine analog is another promising drug currently being studied. The chemical 'NITD008' is the best example[139].

Prevention is the best strategy to treat dengue. At present there are no approved vaccines for dengue. Prevention strategies are aimed at vector control. With rapid urbanisation and people living in more congested localities, the task of prevention is an uphill task and one with a number of roadblocks. It is important to know how to diagnose and treat dengue infection in tropical medicine. In diagnosis, presumptive clinical diagnosis of dengue is, at present, still useful. Further development of new efficient and inexpensive diagnostic tool kits will be useful. In treatment, supportive and symptomatic treatment is the key practice. The ongoing research on antiviral drugs might be the clue to better treatment. Within the next 5 years, dengue will still be a prominent viral infection. The new standardized diagnostic tool kits, including molecular-based, nanodiagnostic and point-of-care testing tool kits will be useful in diagnosis of infection. New antiviral drugs will hopefully become available and aid in the management of dengue infection in the next 5 years.

MATERIALS AND METHODS

Study Design

A simple cross-sectional observational study was conducted in the Outpatient Department (OPD), wards and intensive care unit of the Department of Medicine at Dhiraj Hospital , Pipariya, Waghodia, Vadodara.

Type of Study

Simple cross sectional Observational Study

Sample Size

As Many As Possible (In view of low number of Seropositive case at Dhiraj General Hospital)

Study Period

The study will be done for a period of one and half years.

INCLUSION AND EXCLUSION CRITERIA

Inclusion Criteria

1. Any patient > 18 years of age , male or female, diagnosed as having Dengue fever on basis of
    a. Presence of Antibodies (IgM/IgG) in serum against Dengue fever virus
    b. Testing positive for Dengue NS1 antigen in serum
2. Any patient willing to give written informed consent, fulfilling the above mentioned criteria.

Exclusion Criteria

1. Patients having other co-infections like Malaria, Leptospirosis, Typhoid or Infective Hepatitis, interfering with the interpretation of Laboratory data
2. Patients with known immunocompromised status like patients with HIV
3. Patients below 18 years of age

METHODOLOGY

1. Written informed consent was taken from each patient and study was explained to each patient.
2. Patients were selected based on inclusion/exclusion criteria.
3. Patients of Dengue fever were enrolled from OPD patients or patients admitted to the wards/ICU.
4. Appropriate history was taken and clinical examination of these patients was carried out.
5. Routine investigations like Hb, CBC, RFT's, LFT's, RBS, Serum electrolytes were carried out for each patient from the Central Laboratory.
6. Radiological investigations like Chest X-ray, Ultrasound of the abdomen or thorax, were carried out as per the routine standard followed, based on presentation of the patient. There was no extra cost for these investigations incurred by the patient for being in the study.
7. Invasive investigations like Pleural fluid tapping and Analysis of the fluids were carried out where-ever necessary. Written informed consent of the patient was taken prior to any such investigation.
8. All the data accumulated was compiled properly and conclusions were drawn from the same.

Statistical Methods

Descriptive statistical analysis was carried out in the present study. Results on continuous measurements are presented as Mean ± SD (Min-Max) and results on categorical measurements are presented in number (%). Significance was assessed at 5 % level of significance. Chi square test was used to find the significance of study

parameters on continuous scale between two groups (Inter group analysis). Pearson correlation analysis for finding out correlation between platelets, WBC counts and SGOT/PT was done.

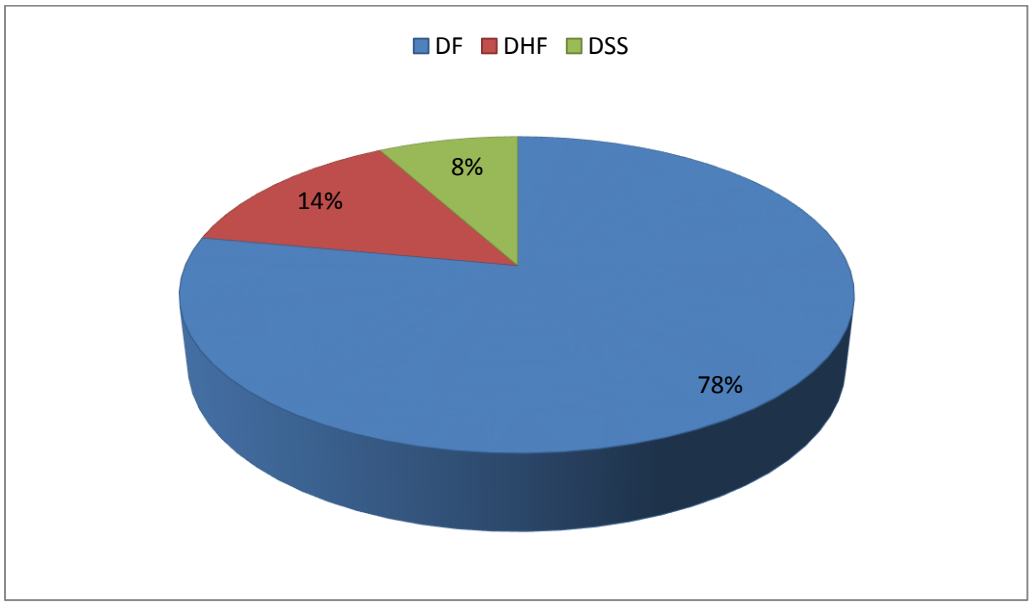
Statistical software

The Statistical software namely SPSS 22.0, Stata 8.0, MedCalc 9.0.1 and Systat 11.0 were used for the analysis of the data and Microsoft Word and Excel have been used to generate graphs, tables etc.

RESULTS

Categories of patients

DIAGNOSIS	Frequency	Percent
DF	39	78.0
DHF	7	14.0
DSS	4	8.0
Total	50	100.0

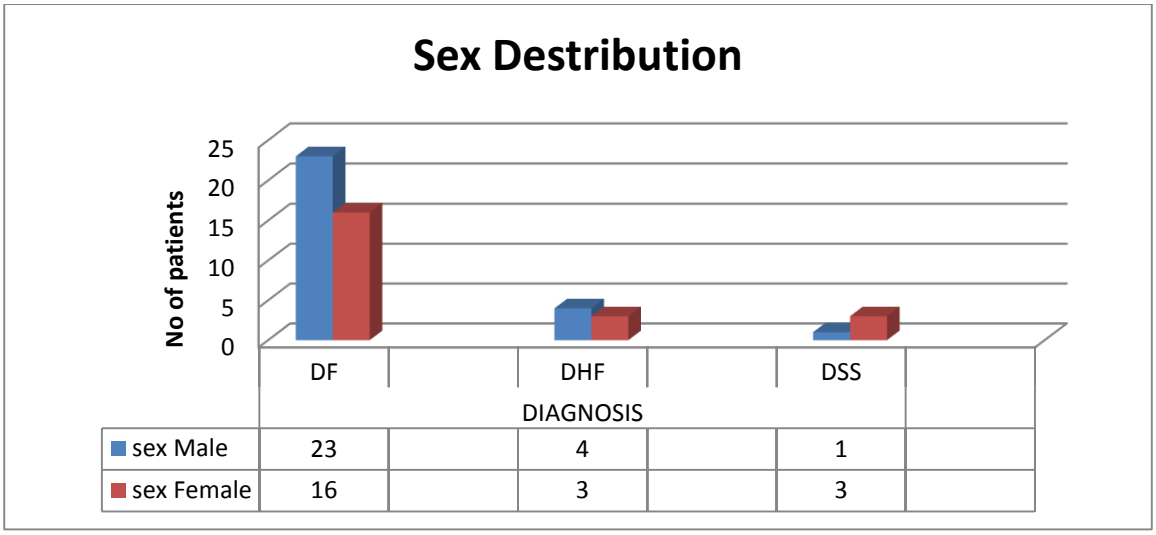


Out of 50 patients 78% had dengue fever, 14% had dengue hemorrhagic fever and 8% had dengue shock syndrome.

Sex distribution

Sex	Frequency	Percent
Male	28	56.0
Female	22	44.0
Total	50	100.0

		sex		Total
		Male	Female	
DIAGNOSIS	DF	23	16	39
		82.1%	72.7%	78.0%
	DHF	4	3	7
		14.3%	13.6%	14.0%
	DSS	1	3	4
		3.6%	13.6%	8.0%
Total		28	22	50
		100.0%	100.0%	100.0%



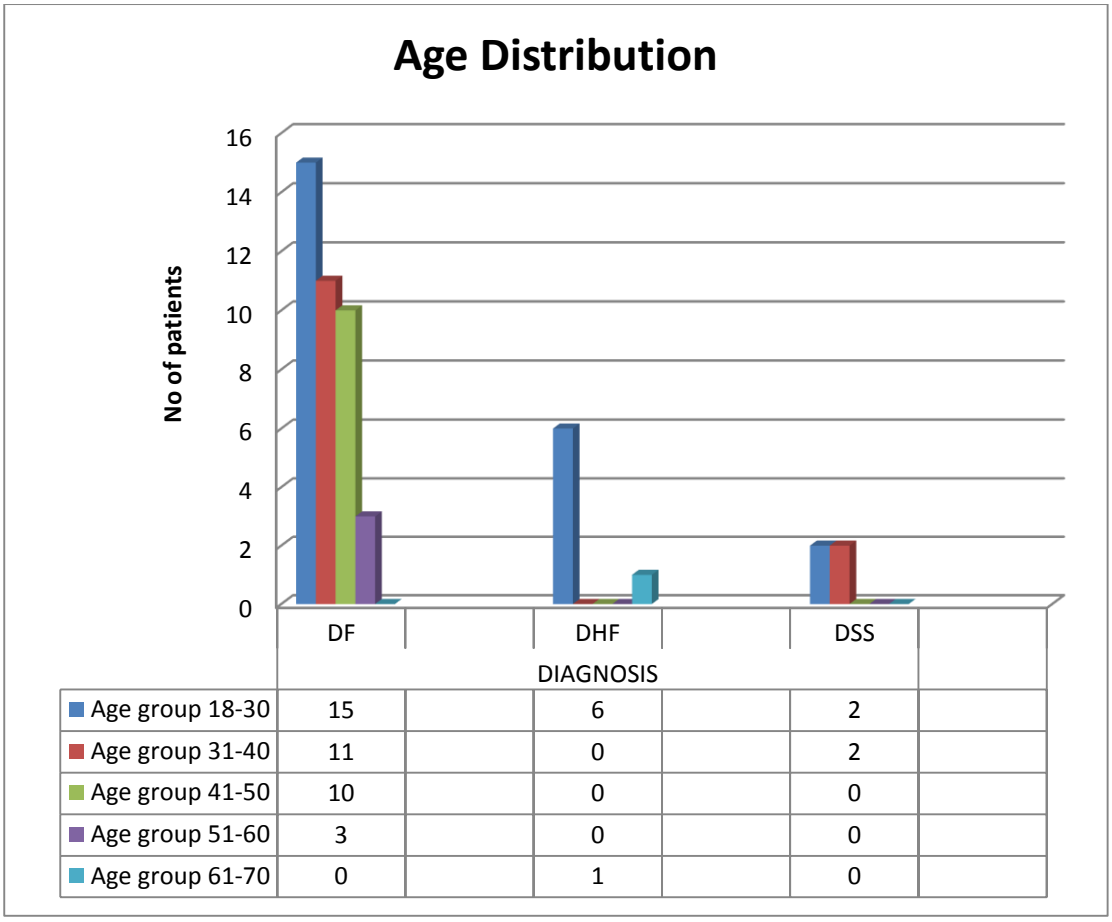
Out of 50 patients, 28 were males and 22 were females.

Age Distribution

	N	Minimum	Maximum	Mean	Std. Deviation
age	50	18	70	32.94	12.154

Age group	Frequency	Percent
18-30	23	46.0
31-40	13	26.0
41-50	10	20.0
51-60	3	6.0
61-70	1	2.0
Total	50	100.0

		Age group					Total
		18-30	31-40	41-50	51-60	61-70	
DIAGNOSIS	DF	15	11	10	3	0	39
		65.2%	84.6%	100.0%	100.0%	.0%	78.0%
	DHF	6	0	0	0	1	7
		26.1%	.0%	.0%	.0%	100.0%	14.0%
	DSS	2	2	0	0	0	4
		8.7%	15.4%	.0%	.0%	.0%	8.0%
Total		23	13	10	3	1	50
		100.0%	100.0%	100.0%	100.0%	100.0%	100.0%



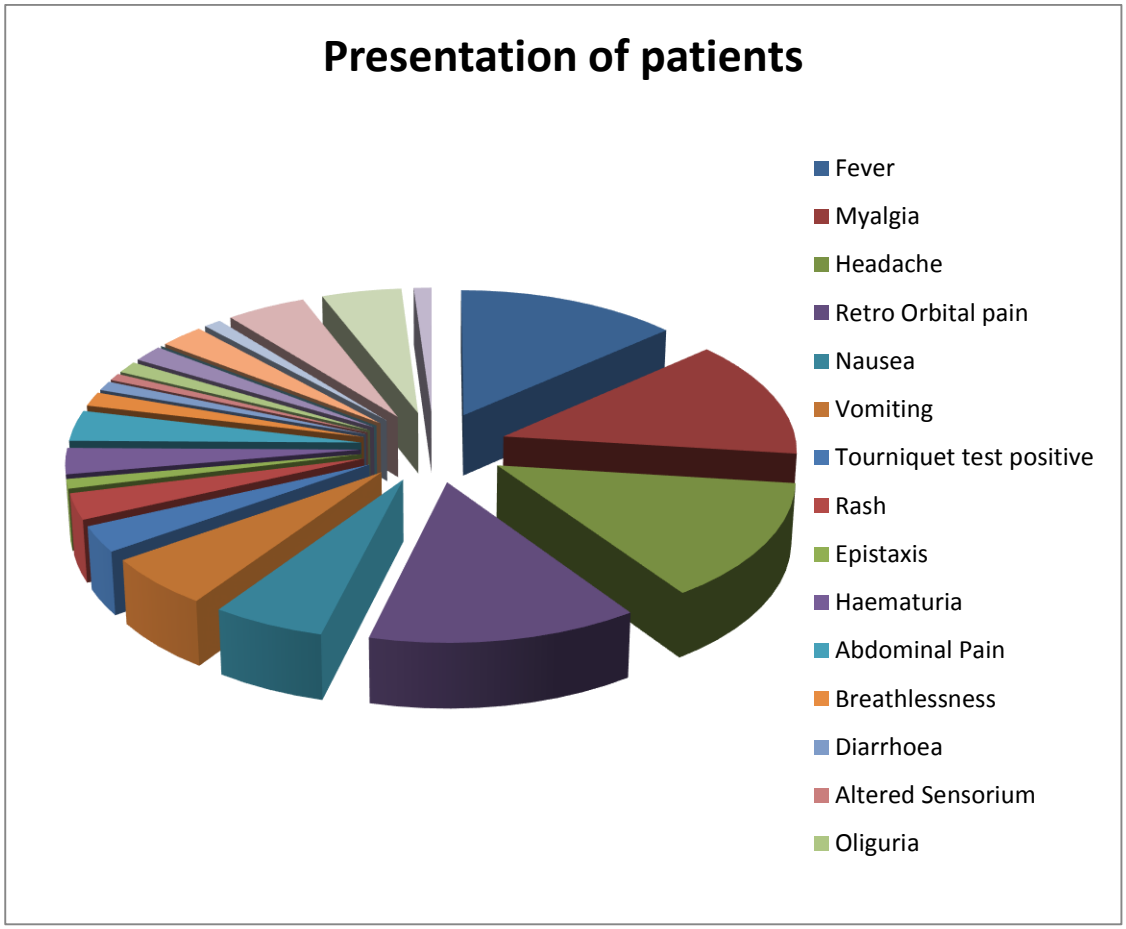
	DF		DHF		DSS	
Age group 18-30	15		6		2	
Age group 31-40	11		0		2	
Age group 41-50	10		0		0	
Age group 51-60	3		0		0	
Age group 61-70	0		1		0	

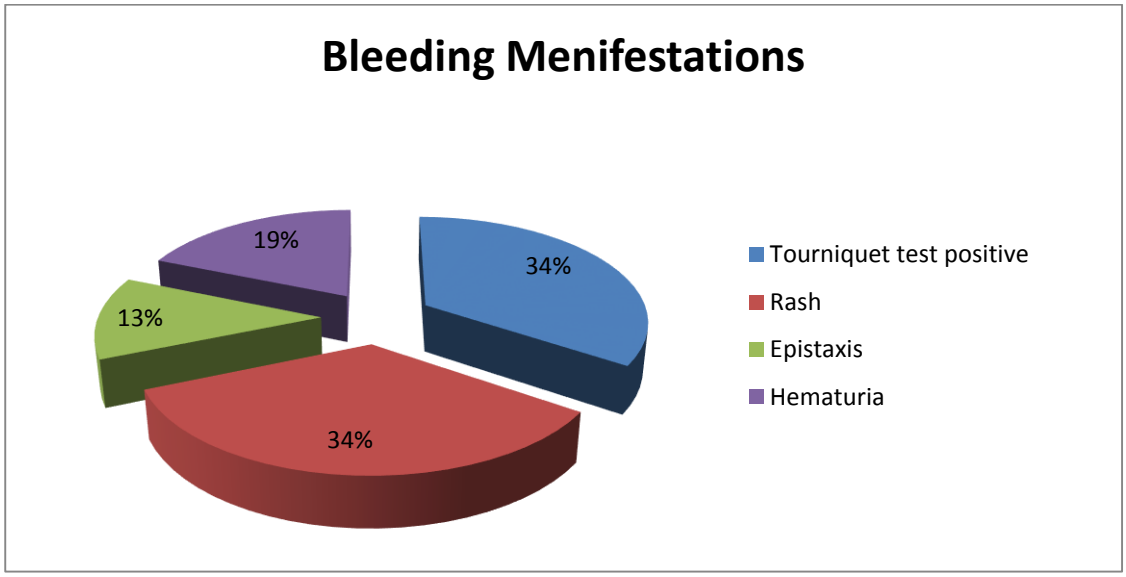
Most patients were of 18-30 years age group (46%) followed by 31-40 years age group, minimum age was 18 and maximum was 70 years.

Presentation of the patients

	No of patients	Percentage
Fever	50	100.0
Myalgia	50	100.0
Headache	50	100.0
Retro Orbital pain	50	100.0
Nausea	21	42.0
Vomiting	21	42.0
Tourniquet test positive	11	22.0
Rash	11	22.0
Epistaxis	4	8.0
Haematuria	11	22.0
Abdominal Pain	13	26.0
Breathlessness	6	12.0
Diarrhoea	4	8.0
Altered Sensorium	3	6.0
Oliguria	5	10.0
Jaundice	7	14.0
Convulsions	0	0
Pleural Effusion	10	20.0
Ascites	4	8.0
Hepatomegaly	18	36.0
Splenomegaly	18	36.0
Hypotension	4	8.0

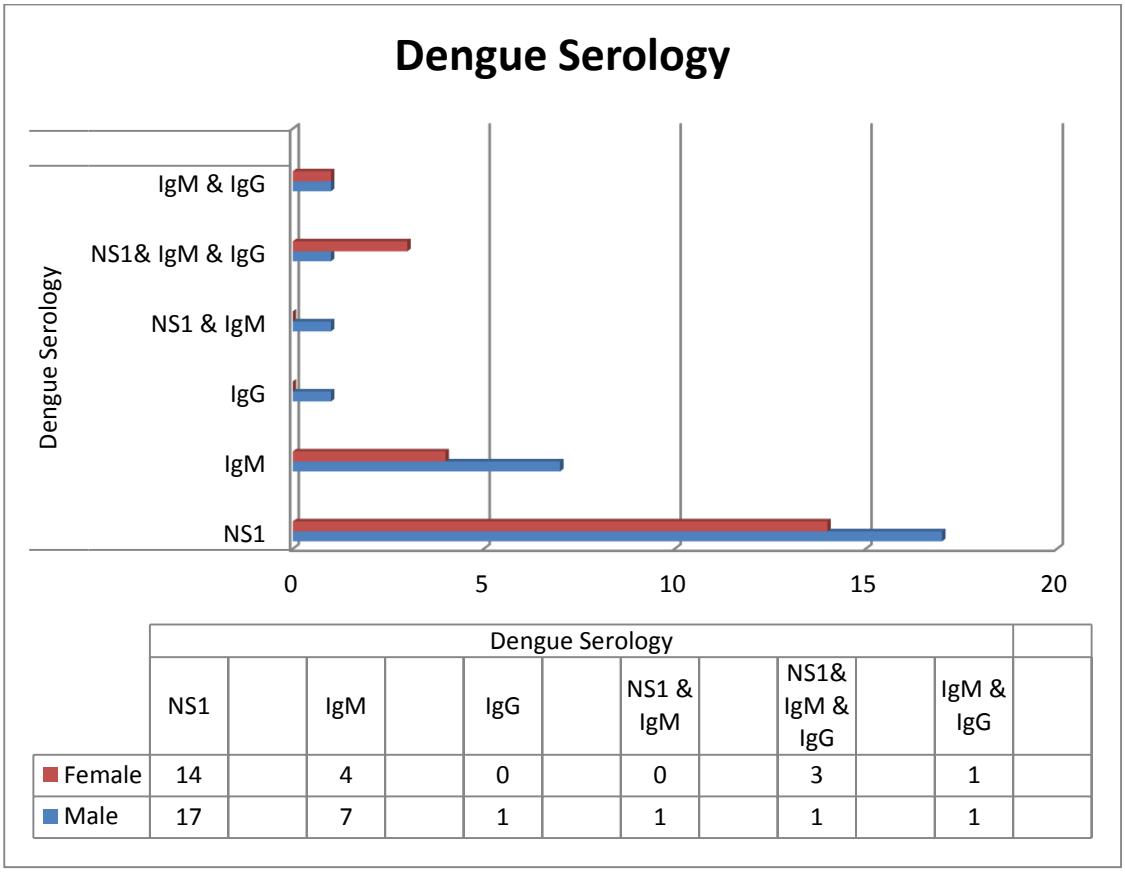
Fever associated with myalgia, headache and retro-orbital pain was the most common presentation present in all the patients in our study.





Rash and positive tourniquet test were the most common bleeding menifestations (34%) followed by haematuria (19%) and epistaxis (13%).

Dengue Serology



	Dengue Serology										
	NS1		IgM		IgG		NS1 & IgM		NS1& IgM & IgG		IgM & IgG	
Female	14		4		0		0		3		1	
Male	17		7		1		1		1		1	

NS1 antigen was the most common to be positive in 31 patients (62%) , IgM and IgG were positive in 11 and 1 patient respectively.

Hematologic Manifestations

Platelets

Descriptive Statistics					
	N	Minimum	Maximum	Mean	Std. Deviation
PLATELETS	50	10000	439000	94000	68949.402

One way ANOVA

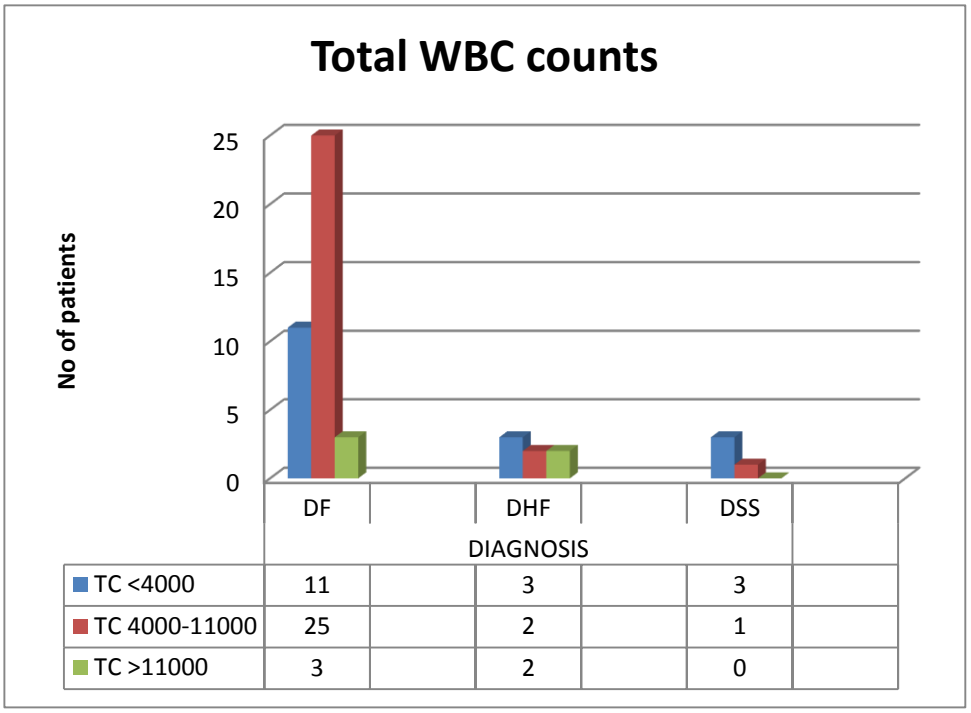
Group	**N**	**Mean Platelets**	**Std. Deviation**	**Std. Error**	**95% Confidence Interval for Mean**	
					Lower Bound	Upper Bound
DF	39	104974.36	71723.331	11484.925	81724.34	128224.37
DHF	7	58571.43	50803.075	19201.757	11586.42	105556.44
DSS	4	49250.00	12737.739	6368.870	28981.41	69518.59
Total	50	94020.00	68949.402	9750.918	74424.80	113615.20

Mean platelet counts were 94000 per cu ml, with lowest count was 10000 per cubic ml.

Total WBC Count

TC	Frequency	Percent
<4000	17	34.0
4000-11000	28	56.0
>11000	5	10.0
Total	50	100.0

		TC			Total
		<4000	4000-11000	>11000	
DIAGNOSIS	DF	11	25	3	39
		28.2%	64.1%	7.7%	100.0%
	DHF	3	2	2	7
		42.9%	28.6%	28.6%	100.0%
	DSS	3	1	0	4
		75.0%	25.0%	.0%	100.0%
Total		17	28	5	50
		34.0%	56.0%	10.0%	100.0%



Among all the patients in the study, 56% had normal total wbc counts, with 10% had leucocytosis and 34% had leucopenia.

Hematocrit

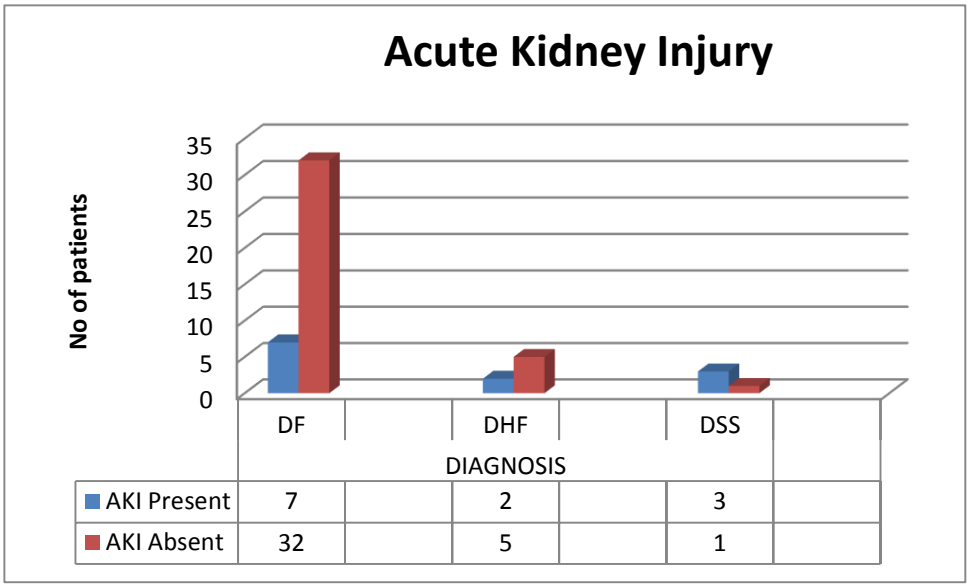
	N	Mean Hematocrit	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
DF	39	38.20	4.938	.791	36.60	39.80
DHF	7	35.66	13.354	5.047	23.31	48.01
DSS	4	42.55	6.553	3.277	32.12	52.98
Total	50	38.19	6.771	.958	36.27	40.11

Mean hematocrit was 38.19 ±1.92 %, increased levels were found in 20 of patients (40%).

Acute Kidney Injury

AKI	Frequency	Percent
Present	12	24.0
Absent	38	76.0
Total	50	100.0

		AKI		Total
		Present	Absent	
DIAGNOSIS	DF	7	32	39
		17.9%	82.1%	100.0%
	DHF	2	5	7
		28.6%	71.4%	100.0%
	DSS	3	1	4
		75.0%	25.0%	100.0%
Total		12	38	50
		24.0%	76.0%	100.0%



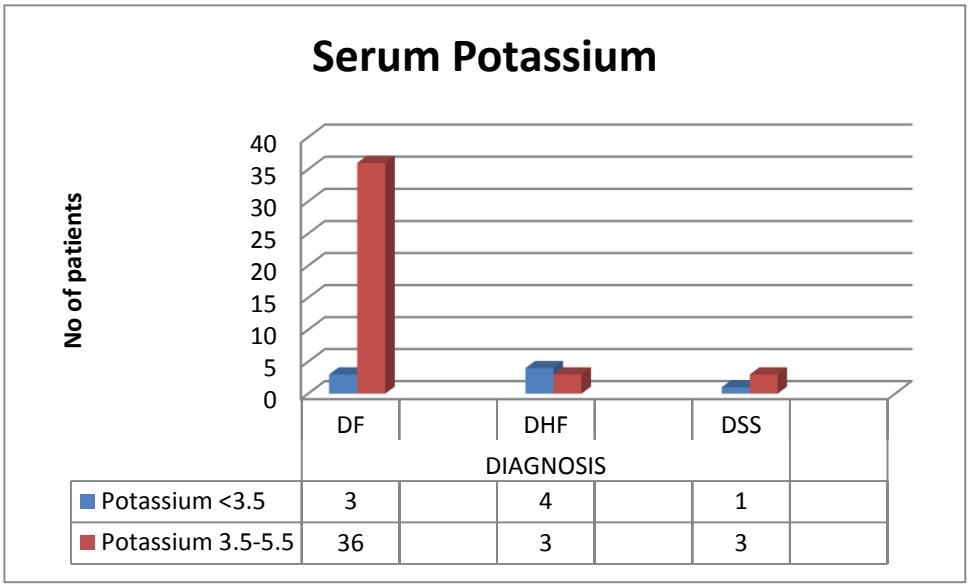
Acute kidney injury (S. Creatinine ≥1.5) was present in 24% of the study patients.

Serum Potassium

Potassium	Frequency	Percent
<3.5	8	16.0
3.5-5.5	42	84.0
Total	50	100.0

Hypokalaemia was present in 16 % of the patients.

		Potassium		Total
		<3.5	3.5-5.5	
DIAGNOSIS	DF	3	36	39
		7.7%	92.3%	100.0%
	DHF	4	3	7
		57.1%	42.9%	100.0%
	DSS	1	3	4
		25.0%	75.0%	100.0%
Total		8	42	50
		16.0%	84.0%	100.0%



Chi-Square Tests			
	Value	df	P-value
Pearson Chi-Square	11.060	2	.004

Liver Function Tests

SGPT

SGPT	Frequency	Percent
Abnormal	14	28.0
Normal (7-56)	36	72.0
Total	50	100.0

		SGPT		Total
		Abnormal	Normal (7-56)	
DIAGNOSIS	DF	7	32	39
		17.9%	82.1%	100.0%
	DHF	3	4	7
		42.9%	57.1%	100.0%
	DSS	4	0	4
		100.0%	.0%	100.0%
Total		14	36	50
		28.0%	72.0%	100.0%

Abnormal SGPT was found in 28% of total patients in this study, with 100% in dengue shock syndrome and 42.9% in dengue hemorrhagic fever patients.

Chi-Square Tests			
	Value	df	P-value
Pearson Chi-Square	13.007	2	.001

SGOT	**Frequency**	**Percent**
Abnormal	31	62.0
Normal (5-40)	19	38.0
Total	50	100.0

		SGOT		**Total**
		Abnormal	Normal (5-40)	
DIAGNOSIS	DF	20	19	39
		51.3%	48.7%	100.0%
	DHF	7	0	7
		100.0%	.0%	100.0%
	DSS	4	0	4
		100.0%	.0%	100.0%
Total		31	19	50
		62.0%	38.0%	100.0%

SGOT was found to be raised in 31 patients with 100% in dengue hemorrhagic and shock syndrome patients.

Chi-Square Tests			
	Value	df	P-value
Pearson Chi-Square	8.644	2	.013

INR (PROTHROMBIN TIME)

INR	Frequency	Percent
1	35	70.0
1-1.5	13	26.0
>1.5	2	4.0
Total	50	100.0

		INR			Total
		1	1-1.5	>1.5	
DIAGNOSIS	DF	33	6	0	39
		84.6%	15.4%	.0%	100.0%
	DHF	2	5	0	7
		28.6%	71.4%	.0%	100.0%
	DSS	0	2	2	4
		.0%	50.0%	50.0%	100.0%
Total		35	13	2	50
		70.0%	26.0%	4.0%	100.0%

No raised INR was found in patient with dengue fever or hemorrhagic fever, but INR was raised in 4% in patient with dengue shock syndrome patient.

Chi-Square Tests			
	Value	df	P-value
Pearson Chi-Square	36.839	4	.000

APTT	**Frequency**	**Percent**
30-40	36	72.0
>40	14	28.0
Total	50	100.0

		APTT		**Total**
		30-40	>40	
DIAGNOSIS	DF	31	8	39
		79.5%	20.5%	100.0%
	DHF	4	3	7
		57.1%	42.9%	100.0%
	DSS	1	3	4
		25.0%	75.0%	100.0%
Total		36	14	50
		72.0%	28.0%	100.0%

APTT was raised in 28% of total patients with being 75% of dengue shock syndrome patients.

Chi-Square Tests			
	Value	df	P-value
Pearson Chi-Square	6.234	2	.044

TOTAL BILIRUBIN

		Total Bilrubin		**Total**
		>1.5	<=1.5	
DIAGNOSIS	DF	1	38	39
		2.6%	97.4%	100.0%
	DHF	3	4	7
		42.9%	57.1%	100.0%
	DSS	3	1	4
		75.0%	25.0%	100.0%
Total		7	43	50
		14.0%	86.0%	100.0%

Total bilirubin was raised in 7 patients in our study (14%), 1 patient in dengue fever group , 3 patients with dengue hemorrhagic fever.

Chi-Square Tests			
	Value	df	P-value
Pearson Chi-Square	21.440	2	<0.001

		Total Bilirubin		**Total**
		>1.5	<=1.5	
sex	Male	1	27	28
		3.6%	96.4%	100.0%
	Female	6	16	22
		27.3%	72.7%	100.0%
Total		7	43	50
		14.0%	86.0%	100.0%

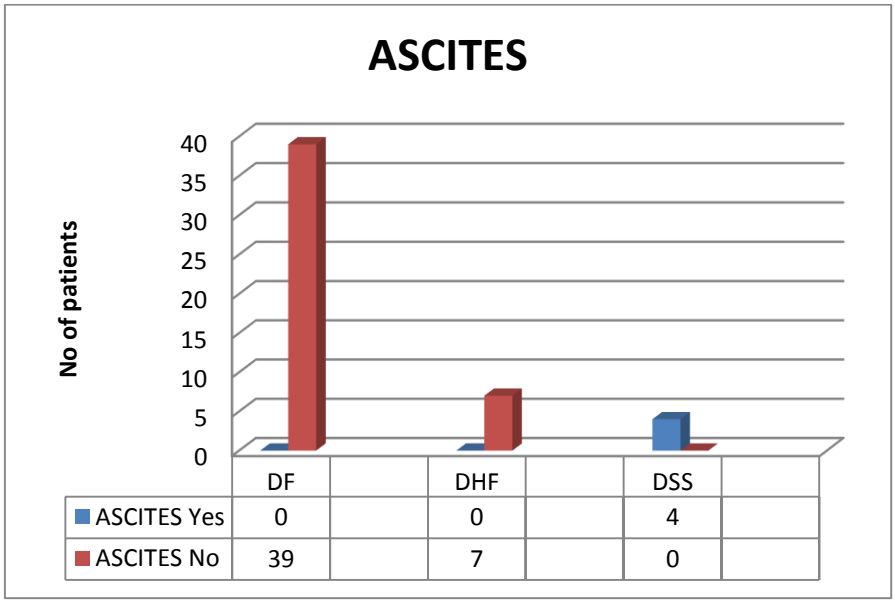
Total bilirubin was raised in 1 male patient and 6 female patients in our study.

ASCITES

		ASCITES		Total
		Yes	No	
Sex	Male	1	27	28
		3.6%	96.4%	100.0%
	Female	3	19	22
		13.6%	86.4%	100.0%
Total		4	46	50
		8.0%	92.0%	100.0%

	Value	df	P-value
Pearson Chi-Square	1.696	1	.193

Ascites was present in 4 patients (8%) which were all patients with dengue shock syndrome.

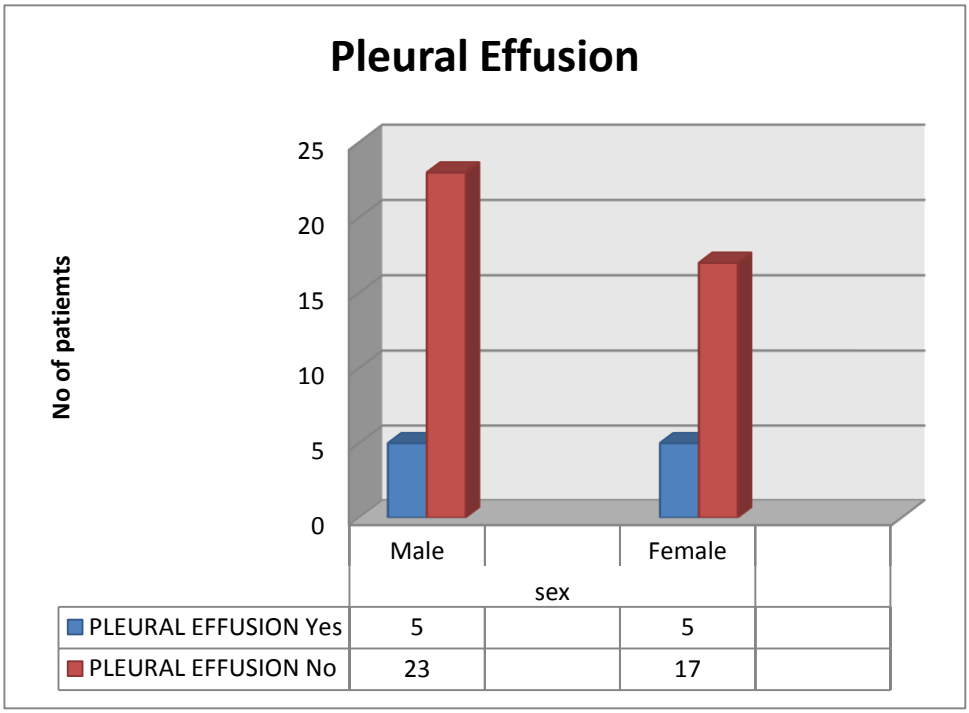


Ascites was present in all shock syndrome patients.

PLEURAL EFFUISON

		PLEURAL EFFUSION		Total
		Yes	No	
sex	Male	5	23	28
		17.9%	82.1%	100.0%
	Female	5	17	22
		22.7%	77.3%	100.0%
Total		10	40	50
		20.0%	80.0%	100.0%

		PLEURAL EFFUSION		Total
		Yes	No	
DIAGNOSIS	DF	0	39	39
		.0%	100.0%	100.0%
	DHF	6	1	7
		85.7%	14.3%	100.0%
	DSS	4	0	4
		100.0%	.0%	100.0%
Total		10	40	50
		20.0%	80.0%	100.0%



Pleural Effusion was present all of DSS patients with 6 patients with dengue hemorrhagic fever patients and overall in 10 patients (20%).

HEPATOMEGALY

		HEPATOMEGALY		Total
		Yes	No	
DIAGNOSIS	DF	7	32	39
		17.9%	82.1%	100.0%
	DHF	7	0	7
		100.0%	.0%	100.0%
	DSS	4	0	4
		100.0%	.0%	100.0%
Total		18	32	50
		36.0%	64.0%	100.0%

Hepatomegaly was present in 18 patients (36%) in our study , being all the patients of dengue hemorrhagic fever and dengue shock syndrome patients, while 7 patients (17.9%) with dengue fever patients.

SPLENOMEGALY

		SPLENOMEGALY		Total
		Yes	No	
DIAGNOSIS	DF	7	32	39
		17.90%	82.10%	100.00%
	DHF	7	0	7
		100.00%	0.00%	100.00%
	DSS	4	0	4
		100.00%	0.00%	100.00%
Total		18	32	50
		36.00%	64.00%	100.00%

Splenomegaly was present in total 18 patients of our study (36%), being all the patients with dengue hemorrhagic fever and dengue shock syndrome, while 7 patients with dengue fever(17.9%)

CORRELATIONS

		Creatinine	Total bilirubin	Sgpt	Sgot	Total Counts
PLATELETS	Pearson Correlation	.197	-.133	-.199	-.234	.342
	P-value	.171	.358	.165	.101	.015
CREATININE	Pearson Correlation		.648	.309	.157	.218
	P-value		.000	.029	.275	.129
	N		50	50	50	50
TOTAL BILIRUBIN	Pearson Correlation			.378	.307	-.056
	P-value			.007	.030	.697
	N			50	50	50
SGPT	Pearson Correlation				.934	.115
	P-value				.000	.425
	N				50	50
SGOT	Pearson Correlation					.058
	P-value					.690
	N					50

Above table shows correlation between Platelets, creatinine, total bilirubin, SGPT, SGOT and total counts. There is weak negative relationship of platelets with creatinine, total bilirubin, SGPT and SGOT but weak positive relation with total counts. Strong positive relationship is seen between Creatinine and total billitubin but there is weak positive relation with other parameters. Total billirubin is positively related with SGPT and SGOT but negatively related with total counts. SGPT and SGOT are strongly related positively. There is weak positive relationship of SGPT and SGOT with total counts.

Oneway ANOVA

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
						Lower Bound	Upper Bound
PLATELETS	DF	39	$1.05x10^5$	71723.331	1.148 $x10^4$	81724.34	128224.37
	DHF	7	5.86 $x10^4$	50803.075	1.920 $x10^4$	11586.42	105556.44
	DSS	4	4.92 $x10^4$	12737.739	6368.870	28981.41	69518.59
	Total	50	9.40 $x10^4$	68949.402	9750.918	74424.80	113615.20
CREATININE	DF	39	1.07	.376	.060	.95	1.19
	DHF	7	1.20	.630	.238	.62	1.78
	DSS	4	2.05	1.406	.703	-.19	4.29
	Total	50	1.17	.592	.084	1.00	1.33
TOTAL BILIRUBIN	DF	39	.83	.333	.053	.72	.93
	DHF	7	1.37	.723	.273	.70	2.04
	DSS	4	6.18	4.046	2.023	-.26	12.61
	Total	50	1.33	1.809	.256	.82	1.84
SGPT	DF	39	40.79	26.879	4.304	32.08	49.51
	DHF	7	108.86	135.113	51.068	-16.10	233.82
	DSS	4	147.75	48.155	24.078	71.12	224.38
	Total	50	58.88	64.804	9.165	40.46	77.30

SGOT	DF	39	52.08	45.870	7.345	37.21	66.95
	DHF	7	187.71	212.816	80.437	-9.11	384.54
	DSS	4	218.00	59.939	29.969	122.62	313.38
	Total	50	84.34	105.881	14.974	54.25	114.43
TOTAL COUNTS	DF	39	5428.21	3004.028	481.029	4454.41	6402.00
	DHF	7	7614.29	5112.543	1932.360	2885.97	12342.60
	DSS	4	3595.00	536.749	268.375	2740.91	4449.09
	Total	50	5587.60	3338.815	472.180	4638.72	6536.48

ANOVA Table

		Sum of Squares	df	Mean Square	F	P-value
PLATELETS	Between Groups	2.149E10	2	1.075×10^{10}	2.389	.103
	Within Groups	2.115E11	47	4.499×10^{9}		
	Total	2.329E11	49			
CREATININE	Between Groups	3.499	2	1.750	6.005	.005
	Within Groups	13.693	47	.291		
	Total	17.192	49			
TOTAL BILIRUBIN	Between Groups	103.829	2	51.914	43.219	.000
	Within Groups	56.456	47	1.201		
	Total	160.285	49			
SGPT	Between Groups	61831.314	2	30915.657	10.094	.000
	Within Groups	143943.966	47	3062.638		
	Total	205775.280	49			
SGOT	Between Groups	186859.022	2	93429.511	12.114	.000
	Within Groups	362474.198	47	7712.217		
	Total	549333.220	49			
TOTAL COUNTS	Between Groups	4.562E7	2	2.281×10^{7}	2.142	.129
	Within Groups	5.006E8	47	1.065×10^{7}		
	Total	5.462E8	49			

Tukey Post Hoc Test for multiple comparison

Dependent Variable	DIAGNOSIS		Mean Difference	Std. Error	P-value
CREATININE	DF	DHF	-.131	.222	.826
		DSS	-.981	.283	.003
	DHF	DF	.131	.222	.826
		DSS	-.850	.338	.040
	DSS	DF	.981	.283	.003
		DHF	.850	.338	.040
TOTAL BILIRUBIN	DF	DHF	-.546	.450	.451
		DSS	-5.349	.575	.000
	DHF	DF	.546	.450	.451
		DSS	-4.804	.687	.000
	DSS	DF	5.349	.575	.000
		DHF	4.804	.687	.000
SGPT	DF	DHF	-68.062	22.717	.012
		DSS	-106.955	29.055	.002
	DHF	DF	68.062	22.717	.012
		DSS	-38.893	34.687	.506
	DSS	DF	106.955	29.055	.002
		DHF	38.893	34.687	.506

SGOT	DF	DHF	-135.637	36.049	.001
		DSS	-165.923	46.106	.002
	DHF	DF	135.637	36.049	.001
		DSS	-30.286	55.044	.847
	DSS	DF	165.923	46.106	.002
		DHF	30.286	55.044	.847

There is significant difference in creatinine, total bilirubin, SGPT and SGOT between DF, DHF and DSS but platelets and total counts are almost same in these diagnosis.

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
PLATELETS	50	10000	439000	94020.00	68949.402
CREATININE	50	0	4	1.17	.592
TOTAL BILIRUBIN	50	0	10	1.33	1.809
SGPT	50	12	413	58.88	64.804
SGOT	50	11	656	84.34	105.881
TOTAL COUNTS	50	2000	15500	5587.60	3338.815

Above table shows summary statistics of creatinine, total bilirubin, SGPT, SGOT, platelets and total counts. Data is summarized in mean and SD.

DISCUSSION

Despite there being a number of studies on dengue fever in the past decade, the incidence of this disease is on the rise. With there being more number of cases reported every year, the need for a study with this design has become necessary. Recent studies point to the trends of dengue fever across various cities in India. There are also multiple reports highlighting the various atypical manifestations of dengue. With more cases being reported, a look into the incidence and also presentations associated with dengue hemorrhagic fever are important, as identifying the same at the earliest and providing for early treatment will change outcome. Also important will be assessing laboratory parameters and involvement of multiple organ systems associated with dengue. Thrombocytopenia is one of the most important parameters to assess patients of dengue fever but its correlation with involvement of other organs has not been studied. Though DSS as an entity is the most severe form of dengue with outcome being adverse in most cases, since it is part of the continuum of DHF with pathophysiology being the same, comparisons made in this study have not considered DSS and DHF as separate entities. In view of the increasing number of cases of dengue fever, this thesis was planned to study clinical and laboratory parameters of dengue fever.

In our study, out of 50 patients, 28 patients were male (56%) and 22 were female (44%) with ratio of 1.2:1, a slight male dominance in our study. In the study conducted by Singh et al in 2003[107] in Delhi the ratio was approximately 3:1, while the study from Surat by Tank Arun G et al[104] had a sex ratio of 2.54:1 in favour of males thereby suggesting the predisposition of males to acquire disease. Also the average age of the patients was 32 years which was the same during the 2003 Delhi

epidemic[107], 26+/-10 years and in a study conducted at Udupi Between 2002-08 by Ashwini Kumar et al[144] had most patients in the age group between 15-44 years. It is not clear why the disease being more common in males and also its higher incidence in young adults.

The most common symptom with which patients presented was Fever , present in all the patients in our study. Average duration of the fever was 4 days. Myalgia and headache were also present. Bleeding manifestations were present in 37 patients (74%). Common bleeding manifestation were rash present in 11 patients (22%), positive tourniquet test and hematuria also present in 11 patients each (22%). Epistaxis was present in 4 patients (8%). In the 1996 outbreak in Delhi as reported by Sharma et al[126] most common presentation was fever and purpura was the most common bleeding manifestation seen in 33.8% of the patients. Also common was hematemesis and melena seen in 22% and 26% patients respectively, unlike this study. Uncommon manifestations seen were diarrhoea (9.3%), altered sensorium (5.3%), jaundice (4%), flaccid quadriparesis (4%) and convulsions (2.7%). Even in the 2003 outbreak of dengue in Delhi[107], altered sensorium, jaundice, pleural effusion and ascites were seen as uncommon manifestations.

Bleeding manifestations were associated with DHF and DSS as compared to dengue fever in our study. This was like the study by R Karoli et al in 2012[106] from Lucknow wherein significant correlation was found between bleeding manifestations and DHF.

Patients in the study were diagnosed on the basis of either NS1 positivity, IgM or IgG positivity. NS1 was positive in 27 patients (54%), while IgM was positive 11 patients (22%) and 1 patient was positive for IgG (2%). There was 1 patients (2%) positive for NS1 and IgM and 2 patients had positive NS1 and IgG (4%), while 4 patients (8%)

had positive IgM, IgG and NS1 antigen. A study from Kolkata on the serodiagnosis of dengue infections found that IgM were positive in more number of patients than NS1 at presentation in the ratio of almost 2:1.[145]

In our study, 46 patients (92%) were found to have thrombocytopenia (<150000/mm^3). The mean platelet count was 94000 per cubic ml. In the study from Delhi in 1996[106], 94 out of 98 patients had thrombocytopenia with mean being 30000/mm^3. Also in the study from Kolkata by Sanjay Kumar Mandal et al[146] in 2013 mean platelet counts were 99000/mm^3.

In our study, 17 patients (34%) presented with leucopenia with mean total counts being 5587/mm^3. 5 patients had leucocytosis (10%). In the study from Delhi in 1996[126], leucopenia was seen in 22 out of 73 patients (30.1%). The hematocrit was raised in 20 patients (40%) with mean being 38.9% in our study. The mean hematocrit was 39.0% in the study from Delhi 1996[126]. Mean hematocrit was 40.9% in the 2013 study from Kolkata by Mandal et al.

There were 12 patients (24%), presented with acute kidney injury in our study. No renal replacement therapy was needed. The mean creatinine of patients in our study was 1.16 mg%. In the 2003 study from Delhi[107], 10 patients (5.4%) presented with AKI out of 185 patients. The mean creatinine in the study by Mandal et al[146] in 2013 was 1.15 mg%. Most patients improved without renal replacement therapy pointing to the pathophysiology of prerenal AKI which improved with hydration.

There were 8 patients (16%) who had potassium levels <3.5 mEq/L, we could not find any correlation of hypokalaemia with dengue.

There were 14 patients (28%) who presented with raised SGPT levels in our study. The mean SGPT level was 58.88 IU/L. While Serum SGOT was raised 31 patients (62%)in our study with mean SGOT was 84.34 IU/L. Serum AST and ALT were elevated in 88% and 76% patients respectively in the 1996 study from Delhi.

Total bilirubin was raised in 7 patients in our study (14%), 1 patient in dengue fever group , 3 patients with dengue hemorrhagic fever.Total bilirubin was raised in 1 male patient and 6 female patients in our study.

No raised INR was found in patient with dengue fever or hemorrhagic fever, but INR was raised in 4% in patient with dengue shock syndrome patient. APTT was raised in 28% of total patients with being 75% of dengue shock syndrome patients.

Ultrasound in the patients in the study was showing Ascites was present in 4 patients (8%) which were all patients with dengue shock syndrome. Pleural Effusion was present all of DSS patients with 6 patients with dengue hemorrhagic fever patients and overall in 10 patients (20%). Tapping was suggestive of transudative effusion. Pleural effusion occurs with dengue as part of serositis in DHF. There we no patients developing ARDS.

Hepatomegaly was present in 18 patients (36%) in our study , being all the patients of dengue hemorrhagic fever and dengue shock syndrome patients, while 7 patients (17.9%) with dengue fever patients

Splenomegaly was present in total 18 patients of our study (36%), being all the patients with dengue hemorrhagic fever and dengue shock syndrome, while 7 patients with dengue fever(17.9%)

No Cardiac manifestations (eg a sinus bradycardia) were seen in patients. Wali et al[128] had reported a case of reversible cardiac dysfunction associated with dengue.

Also no neurological manifestations eg encephalitis, myelitis, GBS have been described in our study.

No patients were expired in our study despite few with very low platelet count or high hematocrit values or bleeding menifestations. The mean hematocrit was significantly elevated to 64% in the study from Delhi in 2003[107] amongst patients who expired.

There were no pregnant females happen to be present in our study.

Above table shows correlation between Platelets, creatinine, total bilirubin, SGPT, SGOT and total counts. There is weak negative relationship of platelets with creatinine, total bilirubin, SGPT and SGOT but weak positive relation with total counts. Strong positive relationship is seen between Creatinine and total bilirubin but there is weak positive relation with other parameters. Total bilirubin is positively related with SGPT and SGOT but negatively related with total counts. SGPT and SGOT are strongly related positively. There is weak positive relationship of SGPT and SGOT with total counts.

There is significant difference in creatinine, total bilirubin, SGPT and SGOT between DF, DHF and DSS but platelets and total counts are almost same in these diagnosis.

Above table shows summary statistics of creatinine, total bilirubin, SGPT, SGOT, platelets and total counts. Data is summarized in mean and SD.

LIMITATIONS OF THE STUDY: There are two potential limitations of our study. First, as it is a cross sectional nature, this study is limited in terms of deriving

mechanistic conclusions; however, results of this study are hypothesis generating and provide insights into the relationship between dengue fever, dengue hemorrhagic fever and other clinical and laboratory parameters. Since all the parameters were recorded only at the time of admission, the progression of the disease, duration of hospital stay and complications that develop during stay could not be commented on. Secondly, the generalizability of this study is limited as the results are limited by number of patients studied.

CONCLUSIONS

1) There is high incidence of dengue fever than dengue hemorrhagic fever or dengue shock syndrome..
2) Males are slight more commonly affected than females in the ratio 1.27:1.
3) Fever associated with myalgia, headache and retro-orbital pain was the most common presentation present in all the patients in our study. Rash and positive tourniquet test were the most common bleeding manifestations (34%) followed by haematuria (19%) and epistaxis (13%).
4) NS1 antigen was the most common to be positive in 31 patients (62%) , IgM and IgG were positive in 11 and 1 patient respectively
5) Mean platelet counts were 94000 per cu ml, with lowest count was 10000 per cubic ml. Among all the patients in the study, 56% had normal total wbc counts, with 10% had leucocytosis and 34% had leucopenia Mean hematocrit was 38.19 ±1.92 %, increased levels were found in 20 of patients (40%).
6) Acute kidney injury (S. Creatinine ≥1.5) was present in 24% of the study patients. Hypokalaemia was present in 16 % of the patients.
7) Abnormal SGPT was found in 28% of total patients in this study, with 100% in dengue shock syndrome and 42.9% in dengue hemorrhagic fever patients. SGOT was found to be raised in 31 patients with 100% in dengue hemorrhagic and shock syndrome patients. Direct hyperbilirubinemia was found in 6% of patients, with 0% in patients with dengue fever while 75% in patients with dengue shock syndrome. APTT was raised in 28% of total patients with being 75% of dengue shock syndrome patients. Total bilirubin was raised in 7 patients in our study (14%), 1 patient in dengue fever group , 3 patients with dengue hemorrhagic fever.

8) Ascites was present in 4 patients (8%) which were all patients with dengue shock syndrome. Pleural Effusion was present all of DSS patients with 6 patients with dengue hemorrhagic fever patients and overall in 10 patients (20%).

9) Hepatomegaly and splenomegaly was present in 18 patients (36%) in our study , being all the patients of dengue hemorrhagic fever and dengue shock syndrome patients, while 7 patients (17.9%) with dengue fever patients.

SUMMARY

In summary, young adults are at risk of dengue the most. Diagnosis of this vector borne disease is usually straight forward and no advanced clinical skill required, this disease has variety of clinical and haematological presentation, with early diagnosis and treatment we can reduce the burden of this disease especially in the endemic countries like India

Further studies will be needed to keep track of the changing epidemiological and clinical trends of dengue. More extensive studies to assess DHF are needed. Also a look into the differences of primary and secondary dengue and their profile is essential.

BIBLIOGRAPHY

1. Chaturvedi UC, Nagar R. Dengue and dengue hemorrhagic fever: Indian perspective. *J. Biosci* 2008;33:429–41
2. WHO. Scientific Working Group Report on Dengue [online]<http://apps.who.int/tdr/publications/tdr-research-publications/swg-report-dengue/pdf/swg_dengue_2.pdf> (WHO, Geneva, Switzerland, 2007).
3. Gubler, D. J. & Meltzer, M. Impact of dengue/dengue hemorrhagic fever on the developing world. *Adv. Virus Res* 1999;53:35–70.
4. WHO. Scientific Working Group Report on Dengue [online]<http://apps.who.int/tdr/publications/tdr-research-publications/swg-report-dengue/pdf/swg_dengue_2.pdf> (WHO, Geneva, Switzerland, 2007). p 3-5.
5. Nvbdcp.gov.in (internet). New Delhi; c2005. (updated 2013 oct 28; cited 2013 oct 28). "dengue cases and deaths in the country since 2007". Available from: http://nvbdcp.gov.in/den-cd.html.
6. Kyle JL, Harris E. Global spread and persistence of Dengue. *Annu Rev Microbiol* 2008;62:71-92
7. Wang E, Ni H, Xu R, Barrett AD, Watowich SJ, Gubler DJ. Evolutionary relationships of endemic/epidemic and sylvatic viruses. *J Virol* Apr 2000; 74:3227-34
8. Rodenhuis-Zybert IA, Wilschut J, Smit JM. Dengue virus life cycle: viral and host factors modulating infectivity. *Cell. Mol. Life Sci* August 2010;67: 2773–86.

9. In: Hanley K.A., Weaver S.C., editors. *Frontiers in Dengue Virus Research*. Poole: Caister Academic Press; 2010.

10. 1k4r: Kuhn R.J., Zhang W., Rossmann M.G., Pletnev S.V., Corver J., Lenches E. et al. Structure of dengue virus: implications for flavivirus organization, maturation, and fusion. *Cell* 2002;108:717-25.

11. 1ok8: Modis, Y., Ogata, S., Clements, D., Harrison, S.C.. Structure of the dengue virus envelope protein after membrane fusion. *Nature* 2004;427:313-9.

12. 1l9k: Egloff, M.P., Benarroch, D., Selisko, B., Romette, J.L., Canard, B. An RNA cap (nucleoside-2'-O-) methyltransferase in the flavivirus RNA polymerase NS5: crystal structure and functional characterization *Embo J* 2002;21:2757-68.

13. 1j7w: Yap, T.L., Xu, T., Chen, Y.L., Malet, H., Egloff, M.P., Canard, B. Crystal Structure of the Dengue Virus RNA-Dependent RNA polymerase Catalytic Domain at 1.85 Angstrom Resolution. *J.Virol* 2007;81:4753.

14. 2vbc: Luo, D.H., Xu, T., Hunke, C., Gruber, G., Vasudevan, S.G., Lescar, J. Crystal Structure of the Ns3 Protease- Helicase from Dengue Virus. *J.Virol* 2008;82:173.

15. Engelthaler DM, Fink TM, Levy CE, Leslie MJ. The re-emergence of *Aedes aegypti* in Arizona. *Emerg Infect Dis* Apr-Jun 1997;3:241-2.

16. Chye JK, Lim CT, Ng KB, Lim JM, George R, Lam SK. Vertical transmission of dengue. *Clin Infect Dis* 1997;25:1374-7.

17. Wagner D, de With K, Huzly D, Hufert F, Weidmann M, Breisinger S, et al. Nosocomial acquisition of dengue. *Emerg Infect Dis* 2004;10:1872-3.

18. Rothman AL. Dengue: defining protective versus pathologic immunity. *J Clin Invest* Apr 2004;113:946-51.

19. Green S, Rothman A. Immunopathological mechanisms in dengue and dengue hemmorhagic fever. *Curr opin Infect Dis* Oct 2006;19:429-36.

20. Rothman AL, Ennis FA. Immunopathogenesis of Dengue hemmorhagic fever. *Virology* Apr 25 1999;257:1-6.

21. Guzman MG, Halstead SB, Artsob H, Buchy P, Farrar J, Gubler DJ, et al. Dengue: a continuing global threat. *Nat Rev Microbiol* 2010 Dec;8:S7-16.

22. Halstead, S. B. Dengue virus–mosquito interactions. *Annu. Rev. Entomol* 2008;53:273–91.

23. Wu, S. J. *et al.* Human skin Langerhans cells are targets of dengue virus infection. *Nature Med* 2000;6:816–20.

24. Jessie, K., Fong, M. Y., Devi, S., Lam, S. K. & Wong, K. T. Localization of dengue virus in naturally infected human tissues, by immunohistochemistry and *in situ* hybridization. *J. Infect. Dis* 2004;189:1411–8.

25. Halstead, S. B. & O'Rourke, E. J. Dengue viruses and mononuclear phagocytes. I. Infection enhancement by non‐neutralizing antibody. *J. Exp. Med* 1977;146:201–17.

26. Halstead, S. B. *In vivo* enhancement of dengue virus infection in rhesus monkeys by passively transferred antibody. *J. Infect. Dis* 1979;140:527–33.

27. Goncalvez, A. P., Engle, R. E., St. Claire, M., Purcell, R. H. & Lai, C. J. Monoclonal antibody mediated enhancement of dengue virus infection *in vitro* and *in vivo* and strategies for prevention. *Proc. Natl Acad. Sci USA* 2007;104: 9422–7.

28. Kliks, S. C., Nimmanitya, S., Nisalak, A. & Burke, D. S. Evidence that maternal dengue antibodies are important in the development of dengue hemorrhagic fever in infants. *Am. J. Trop. Med. Hyg* 1988;38:411–9.

29. Cummings, D. A. T., Schwartz, I. B., Billings, L., Shaw, L. B. & Burke, D. S. Dynamic effects of antibody‐dependent enhancement on the fitness of viruses. *Proc. Natl Acad. Sci. USA* 2005;102:15259–64.

30. Martina B.E, Koraka P, Osterhaus A.D. Dengue virus pathogenesis: an integrated view. *Clin Microbiol Rev* 2009 Oct;22(4):564-81.

31. Limon-Flores A. Y., M. Perez-Tapia, I. Estrada-Garcia, G. Vaughan, A. Escobar-Gutierrez, J. Calderon-Amador et al. Dengue virus inoculation to human skin explants: an effective approach to assess in situ the early infection and the effects on cutaneous dendritic cells. *Int. J. Exp. Pathol* 2005; 86:323–34.

32. Wu S. J., G. Grouard-Vogel, W. Sun, J. R. Mascola, E. Brachtel, R. Putvatana et al. Human skin Langerhans cells are targets of dengue virus infection. *Nat. Med* 2000;6:816–20.

33. Durbin, A. P., M. J. Vargas, K. Wanionek, S. N. Hammond, A. Gordon, C. Rocha, A. Balmaseda, and E. Harris. Phenotyping of peripheral blood mononuclear cells during acute dengue illness demonstrates infection and increased activation of monocytes in severe cases compared to classic dengue fever. *Virology* 2008;376:429–35.

34. Boonnak K., B. M. Slike, T. H. Burgess, R. M. Mason, S. J. Wu, P. Sun et al. Role of dendritic cells in antibody-dependent enhancement of dengue virus infection. *J. Virol* 2008;82:3939–51.

35. Ho, L. J., M. F. Shaio, D. M. Chang, C. L. Liao, and J. H. Lai. Infection of human dendritic cells by dengue virus activates and primes T cells towards

Th0-like phenotype producing both Th1 and Th2 cytokines. *Immunol. Investig* 2004;33:423–37.

36. Blackley S., Z. Kou, H. Chen, M. Quinn, R. C. Rose, J. J. Schlesinger et al. Primary human splenic macrophages, but not T or B cells, are the principal target cells for dengue virus infection in vitro. *J. Virol* 2007;81:13325–34.

37. Huerre M. R., N. T. Lan, P. Marianneau, N. B. Hue, H. Khun, N. T. Hung et al. Liver histopathology and biological correlates in five cases of fatal dengue fever in Vietnamese children. *Virchows Arch* 2001; 438:107–15.

38. Kyle J. L., P. R. Beatty, and E. Harris. Dengue virus infects macrophages and dendritic cells in a mouse model of infection. *J. Infect. Dis* 2007;195:1808–17.

39. Balsitis S. J., J. Coloma, G. Castro, A. Alava, D. Flores, J. H. McKerrow. Tropism of dengue virus in mice and humans defined by viral nonstructural protein 3-specific immunostaining. *Am. J. Trop. Med. Hyg* 2009;80:416–24.

40. Basilio-de-Oliveira C. A., G. R. Aguiar, M. S. Baldanza, O. M. Barth, W. A. Eyer-Silva, M. V. Paes. Pathologic study of a fatal case of dengue-3 virus infection in Rio de Janeiro, Brazil. *Braz. J. Infect. Dis* 2005;9:341–7.

41. Kangwanpong D., N. Bhamarapravati, H. L. Lucia. Diagnosing dengue virus infection in archived autopsy tissues by means of the in situ PCR method: a case report. *Clin. Diagn. Virol* 1995;3:165–72.

42. Avirutnan P., P. Malasit, B. Seliger, S. Bhakdi, M. Husmann. Dengue virus infection of human endothelial cells leads to chemokine production, complement activation, and apoptosis. *J. Immunol* 1998;161:6338– 46.

43. Bethell D. B., J. Gamble, P. L. Pham, M. D. Nguyen, T. H. Tran, T. H. Ha et al. Noninvasive measurement of microvascular leakage in patients with dengue hemorrhagic fever. *Clin. Infect. Dis* 2001;32:243–53.

44. Cardier J. E., E. Marino, E. Romano, P. Taylor, F. Liprandi, N. Bosch et al. Proinflammatory factors present in sera from patients with acute dengue infection induce activation and apoptosis of human microvascular endothelial cells: possible role of TNF-alpha in endothelial cell damage in dengue. *Cytokine* 2005;30:359–65.

45. Chen, H. C., F. M. Hofman, J. T. Kung, Y. D. Lin, B. A. Wu-Hsieh. Both virus and tumor necrosis factor alpha are critical for endothelium damage in a mouse model of dengue virus-induced hemorrhage. *J. Virol* 2007;81:5518–26.

46. Butthep P., S. Chunhakan, K. Tangnararatchakit, S. Yoksan, K. Pattanapanyasat, A. Chuansumrit. Elevated soluble thrombomodulin in the febrile stage related to patients at risk for dengue shock syndrome. *Pediatr. Infect. Dis. J* 2006;25:894–7.

47. Cardier J. E., B. Rivas, E. Romano, A. L. Rothman, C. Perez-Perez, M. Ochoa et al. Evidence of vascular damage in dengue disease: demonstration of high levels of soluble cell adhesion molecules and circulating endothelial cells. *Endothelium* 2006;13:335–40.

48. Avirutnan P., L. Zhang, N. Punyadee, A. Manuyakorn, C. Puttikhunt, W. Kasinrerk et al. Secreted NS1 of dengue virus attaches to the surface of cells via interactions with heparan sulfate and chondroitin sulfate E. *PLoS Pathog* 2007;3:e183.

49. Guzman M. G., and G. Kouri. Dengue and dengue hemorrhagic fever in the Americas: lessons and challenges. *J. Clin. Virol* 2003;27:1–13.

50. Halstead, S. B. Dengue. *Lancet* 2007;370:1644–52.

51. Ong A., M. Sandar, M. I. Chen, L. Y. Sin. Fatal dengue hemorrhagic fever in adults during a dengue epidemic in Singapore. *Int. J. Infect. Dis* 2007; 11:263–7.

52. Cologna R, R. Rico-Hesse. American genotype structures decrease dengue virus output from human monocytes and dendritic cells. *J. Virol* 2003; 77:3929–38.

53. Churdboonchart V., N. Bhamarapravati, P. Futrakul. Crossed immunoelectrophoresis for the detection of split products of the third complement in dengue hemorrhagic fever. I. Observations in patients' plasma. *Am. J. Trop. Med. Hyg* 1983;32:569–76.

54. Nishioka, K. Serum complement level in dengue hemorrhagic fever. *Allerg. Immunol. (Leipz)* 1974;21:385–92.

55. Kurosu T., P. Chaichana, M. Yamate, S. Anantapreecha, K. Ikuta. Secreted complement regulatory protein clusterin interacts with dengue virus nonstructural protein 1. *Biochem. Biophys. Res. Commun* 2007;362:1051–6.

56. Koraka, P., C. Suharti, T. E. Setiati, A. T. Mairuhu, E. Van Gorp, C. E. Hack et al. Kinetics of dengue virus-specific serum immunoglobulin classes and subclasses correlate with clinical outcome of infection. *J. Clin. Microbiol* 2001; 39:4332–8.

57. Thein S., J. Aaskov, T. T. Myint, T. N. Shwe, T. T. Saw, A. Zaw. Changes in levels of anti-dengue virus IgG subclasses in patients with disease of varying severity. *J. Med. Virol* 1993;40:102–6.

58. Chungue E., L. Poli, C. Roche, P. Gestas, P. Glaziou, L. J. Markoff.s. Correlation between detection of plasminogen cross-reactive antibodies and hemorrhage in dengue virus infection. *J. Infect. Dis* 1994;170:1304–7.

59. Libraty D. H., P. R. Young, D. Pickering, T. P. Endy, S. Kalayanarooj, S. Green et al. High circulating levels of the dengue virus nonstructural protein NS1 early in dengue illness correlate with the development of dengue hemorrhagic fever. *J. Infect. Dis* 2002;186:1165–8.

60. Loke, H., D. Bethell, C. X. Phuong, N. Day, N. White, J. Farrar et al. Susceptibility to dengue hemorrhagic fever in Vietnam: evidence of an association with variation in the vitamin d receptor and Fc gamma receptor IIa genes. *Am. J. Trop. Med. Hyg* 2002;67:102–6.

61. Chao, Y. C., C. S. Huang, C. N. Lee, S. Y. Chang, C. C. King, C. L. Kao. Higher infection of dengue virus serotype 2 in human monocytes of patients with G6PD deficiency. *PLoS One* 2008;3:e1557

62. Acioli-Santos B., L. Segat, R. Dhalia, C. A. Brito, U. M. Braga-Neto, E. T. Marques et al. MBL2 gene polymorphisms protect against development of thrombocytopenia associated with severe dengue phenotype. *Hum. Immunol* 2008;69:122–8.

63. Chen R. F., L. Wang, J. T. Cheng, H. Chuang, J. C. Chang, J. W. Liu et al. Combination of CTLA-4 and TGFbeta1 gene polymorphisms associated with dengue hemorrhagic fever and virus load in a dengue-2 outbreak. *Clin. Immunol* 2009;131:404–9.

64. Fernandez-Mestre M. T., K. Gendzekhadze, P. Rivas-Vetencourt, Z. Layrisse. TNF-alpha-308A allele, a possible severity risk factor of hemorrhagic manifestation in dengue fever patients. *Tissue Antigens* 2004;64:469–72.

65. Soundravally R., S. L. Hoti. Immunopathogenesis of dengue hemorrhagic fever and shock syndrome: role of TAP and HPA gene polymorphism. *Hum. Immunol* 2007;68:973–9.

66. Sakuntabhai A., C. Turbpaiboon, I. Casademont, A. Chuansumrit, T. Lowhnoo, A. Kajaste-Rudnitski et al. A variant in the CD209 promoter is associated with severity of dengue disease. *Nat. Genet* 2005;37:507–13.

67. Loke H., D. B. Bethell, C. X. Phuong, M. Dung, J. Schneider, N. J. White et al. Strong HLA class I-restricted T cell responses in dengue hemorrhagic fever: a double-edged sword? *J. Infect. Dis* 2001;184:1369–73.

68. Ghiasi H., G. C. Perng, A. B. Nesburn, S. L. Wechsler. Antibody dependent enhancement of HSV-1 infection by anti-gK sera. *Virus Res* 2000;68:137–44.

69. Girn, J., M. Kavoosi, J. Chantler. Enhancement of coxsackievirus B3 infection by antibody to a different coxsackievirus strain. *J. Gen. Virol* 2002;83:351–8.

70. Graham R. R., M. Juffrie, R. Tan, C. G. Hayes, I. Laksono, C. Ma'roef et al. A prospective sero-epidemiologic study on dengue in children four to nine years of age in Yogyakarta, Indonesia I. studies in 1995–1996. *Am. J. Trop. Med. Hyg* 1999;61:412–9.

71. Halstead, S. B. Observations related to pathogensis of dengue hemorrhagic fever. VI. Hypotheses and discussion. *Yale J. Biol. Med* 1970;42:350–62.

72. Libraty D. H., T. P. Endy, H. S. Houng, S. Green, S. Kalayanarooj, S. Suntayakorn et al. Differing influences of virus burden and immune activation on disease severity in secondary dengue-3 virus infections. *J. Infect. Dis* 2002;185:1213–21.

73. Lidbury B. A., S. Mahalingam. Specific ablation of antiviral gene expression in macrophages by antibody-dependent enhancement of Ross River virus infection. *J. Virol* 2000;74:8376–81.

74. Mahalingam S., B. A. Lidbury. Suppression of lipopolysaccharide induced antiviral transcription factor (STAT-1 and NF-kappa B) complexes by

antibody-dependent enhancement of macrophage infection by Ross River virus. *Proc. Natl. Acad. Sci. USA* 2002;99:13819–24.

75. Chanthavanich P., C. Luxemburger, C. Sirivichayakul, K. Lapphra, K. Pengsaa, S. Yoksan et al. Immune response and occurrence of dengue infection in Thai children three to eight years after vaccination with live attenuated tetravalent dengue vaccine. *Am. J. Trop. Med. Hyg* 2006;75:26–8.

76. An, J., D. S. Zhou, J. L. Zhang, H. Morida, J. L. Wang, K. Yasui. Dengue-specific $CD8^+$ T cells have both protective and pathogenic roles in dengue virus infection. *Immunol. Lett* 2004;95:167–74.

77. Mongkolsapaya J., T. Duangchinda, W. Dejnirattisai, S. Vasanawathana, P. Avirutnan, A. Jairungsri et al. T cell responses in dengue hemorrhagic fever: are cross-reactive T cells suboptimal? *J. Immunol* 2006;176:3821–9.

78. Dong T., E. Moran, N. Vinh Chau, C. Simmons, K. Luhn, Y. Peng et al. High pro-inflammatory cytokine secretion and loss of high avidity cross-reactive cytotoxic T-cells during the course of secondary dengue virus infection. *PLoS One* 2007;2:e1192.

79. Imrie A., J. Meeks, A. Gurary, M. Sukhbataar, P. Kitsutani, P. Effler et al. Differential functional avidity of dengue virus-specific T-cell clones for variant peptides representing heterologous and previously encountered serotypes. *J. Virol* 2007;81:10081–91.

80. Mongkolsapaya J., W. Dejnirattisai, X. N. Xu, S. Vasanawathana, N.Tangthawornchaikul, A. Chairunsri et al. Original antigenic sin and apoptosis in the pathogenesis of dengue hemorrhagic fever. *Nat. Med* 2003;9:921–7.

81. Klenerman P., R. M. Zinkernagel. Original antigenic sin impairs cytotoxic T lymphocyte responses to viruses bearing variant epitopes. *Nature* 1998;394:482–5.

82. Cornberg M., A. T. Chen, L. A. Wilkinson, M. A. Brehm, S. K. Kim, C. Calcagno et al. Narrowed TCR repertoire and viral escape as a consequence of heterologous immunity. *J. Clin. Investig* 2006;116:1443–56.

83. Azeredo E. L., S. M. Zagne, M. A. Santiago, A. S. Gouvea, A. A. Santana, P. C. Neves-Souza, R. M. Nogueira, M. P. Miagostovich, and C. F. Kubelka. Characterisation of lymphocyte response and cytokine patterns in patients with dengue fever. *Immunobiology* 2001;204:494–507.

84. Bozza F. A., O. G. Cruz, S. M. Zagne, E. L. Azeredo, R. M. Nogueira, E. F. Assis et al. Multiplex cytokine profile from dengue patients: MIP-1beta and IFN-gamma as predictive factors for severity. *BMC. Infect. Dis* 2008;8:86.

85. Chakravarti A., R. Kumaria. Circulating levels of tumour necrosis factor-alpha & interferon-gamma in patients with dengue & dengue hemorrhagic fever during an outbreak. *Indian J. Med. Res* 2006;123:25–30.

86. Cardier J. E., V. Balogh, C. Perez-Silva, E. Romano, B. Rivas, N. Bosch et al. Relationship of thrombopoietin and interleukin- 11 levels to thrombocytopenia associated with dengue disease. *Cytokine* 2006;34:155–60.

87. Atrasheuskaya A., P. Petzelbauer, T. M. Fredeking, G. Ignatyev. Anti-TNF antibody treatment reduces mortality in experimental dengue virus infection. *FEMS Immunol. Med. Microbiol* 2003;35:33–42.

88. Lin C. F., S. C. Chiu, Y. L. Hsiao, S. W. Wan, H. Y. Lei, A. L. Shiau et al. Expression of cytokine, chemokine, and adhesion molecules during

endothelial cell activation induced by antibodies against dengue virus nonstructural protein 1. *J. Immunol* 174:395–403.

89. Yamada M., S. Kim, K. Egashira, M. Takeya, T. Ikeda, O. Mimura et al. Molecular mechanism and role of endothelial monocyte chemoattractant protein-1 induction by vascular endothelial growth factor. *Arterioscler. Thromb. Vasc. Biol* 2003;23:1996–2001.

90. Agarwal, R., U. C. Chaturvedi, A. Misra, R. Mukerjee, S. Kapoor, R. Nagar, R. Tandon, A. Mathur. Production of cytotoxic factor by peripheral blood mononuclear cells (PBMC) in patients with dengue hemorrhagic fever. *Clin. Exp. Immunol* 1998;112:477–81.

91. Chaturvedi, U. C., E. A. Elbishbishi, R. Agarwal, A. S. Mustafa. Cytotoxic factor-autoantibodies: possible role in the pathogenesis of dengue hemorrhagic fever. *FEMS Immunol. Med. Microbiol* 2001;30:181–6.

92. Medscape.com (internet). New York: WebMD LLC; c1994-2013. (cited 2013 Nov 28). Available from http://emedicine.medscape.com/article/215840-clinical

93. Whitehorn J, Farrar J. Dengue. *Br. Med. Bull* 2010;95:161–73.

94. WHO. Scientific Working Group Report on Dengue [online]<http://apps.who.int/tdr‑publications/tdr‑research‑publications/swg‑report‑dengue/pdf/swg_dengue_2.pdf> (WHO, Geneva, Switzerland, 2007). p 14-6.

95. Reiter P. Yellow fever and dengue: a threat to Europe?. *Euro Surveill* 2010;15:19509.

96. Simmons CP, Farrar JJ, Nguyen vV, Wills B. Dengue. *N Engl J Med* 2012;366:1423–32.

97. Chen LH, Wilson ME. Dengue and chikungunya infections in travelers. *Current Opinion in Infectious Diseases* 2010;23:438–44.

98. Seth W. Wright, Meg Jack. Tropical medicine. In: Knoop KJ, Stack LB, Storrow A, Thurman RJ, editors. *Atlas of emergency medicine,* 3rd ed. New York: McGraw-Hill Professional. 2010;p.658–9.

99. WHO. Scientific Working Group Report on Dengue [online]<http://apps. who.int/tdr/publications/tdr‐research‐publications/swg‐report‐dengue/pdf/swg_dengue_2.pdf> (WHO, Geneva, Switzerland, 2007). p 25-7.

100. Ranjit S, Kissoon N. Dengue hemorrhagic fever and shock syndromes. *Pediatr. Crit. Care Med* 2011;12:90–100.

101. Rodenhuis-Zybert IA, Wilschut J, Smit JM. Dengue virus life cycle: viral and host factors modulating infectivity. *Cell. Mol. Life Sci* 2010;67:2773–86.

102. Dengue hemorrhagic fever: diagnosis, treatment, prevention and control. 2nd edition. Geneva : World Health Organization. c1997- (cited 2013 nov 28). Available from http://www.who.int/csr/ resources/ publications/dengue /Denguepublication/en/

103. Victor TJ, Malathi M, Gurusamy D, Desai A, Ravi V, Narayanasamy G et al. Dengue fever outbreaks in two villages of Dharmapuri district in Tamil Nadu. *Indian J Med Res* 2002 Oct;116:133-9.

104. Tank Arun G, Jain Mannu R. Trend of Dengue in a tertiary care hospital of Surat city, Western India. *National journal of community medicine* Apr-jun 2012;3:302-4.

105. Ragini Mishra, Prashant Kumar. A study on outbreak of Dengue from Bihar, India- establishing new foci attributable to climatic change. *Journal of public health and epidemiology* Nov 2011;3:489-502.

106. Karoli R, Fatima J, Siddiqi Z, Kazmi KI, Sultania AR. Clinical profile of dengue infection at a teacing hospital in North India. *J Infect Dev Ctries* 2012 Jul 23;6:551-4.

107. Singh NP, Jhamb R, Agarwal SK, Gaiha M, Dewan R, Daga MK et al. The 2003 outbreak of dengue fever in Delhi, India. *Southeast Asian J Trop Med Public Health* 2005 Sep;36:1174-8.

108. Gulati S, Maheshwari A. Atypical manifestations of dengue. *Trop Med Int Health* 2007 Sep;12:1087-95.

109. Medscape.com (internet). New York: WebMD LLC; c1994-2013. (cited 2013 Nov 28). Available from http://emedicine.medscape.com/article/215840-workup

110. Trung DT, Thao le TT, Hien TT, et al. Liver involvement associated with dengue infection in adults in Vietnam. *Am J Trop Med Hyg* Oct 2010;83:774-80.

111. Oliveira, Éveny Cristine Luna de et al. Haematological abnormalities in patients with dengue. *Rev. Soc. Bras. Med. Trop* 2009 Nov-Dec;42:682-5.

112. Potts JA, Rothman AL. Clinical and laboratory features that distinguish dengue from other febrile illnesses in endemic populations. *Trop Med Int Health* Nov 2008;13:1328-40.

113. Lin SF, Liu HW, Chang CS, Yen JH, Chen TP. Haematological aspects of dengue fever. *Gaoxiong Yi Xue Ke Xue Za Zhi* 1989 Jan;5:12-6.

114. Souza LJ, Alves JG, Nogueira RM, Gicovate Neto C, Bastos DA, Siqueira EW et al. Aminotransferase changes and acute hepatitis in patients with dengue fever: analysis of 1585 cases. *Braz J Infect Dis* 2004 Apr;8:156-63.

115. Muhammad A.M. Khalil, Sarfaraz Sarwar, Muhammad A. Chaudry, Baila Maqbool, Zarghoona Khalil, Jackson Tan et al. Acute kidney injury in dengue virus infection. *Clin Kidney J* 2012;5:390–4.

116. Ing-Kit Lee, Jien-Wei Liu, Kuender D. Yang. Clinical Characteristics, Risk Factors, and Outcomes in Adults Experiencing DengueHemorrhagic Fever Complicated with Acute Renal Failure. *Am. J. Trop. Med. Hyg* 2009;80:651–5.

117. Mehra N, Patel A, Abraham G, Reddy YN, Reddy YN. Acute kidney injury in dengue fever using Acute Kidney Injury network criteria: incidence ad risk factors. *Trop Doct* 2012 Jul;42:160-2.

118. Hutspardol S, Prommalikit O, Upiya N, Chataroopwijit J, Khemakanok K, Assadamongkol K. Heavy proteinuria following dengue hemmorhagic fever. *Southeast Asian J Trop Med Public Health* 2011 May;42:579-82.

119. Joshi R, Baid V. Profile of dengue patients admitted to a tertiary care hospital in Mumbai. *Turk J Pediatr* 2011 Nov-Dec;53:626-31.

120. Kanade T, Shah I. Dengue encephalopathy. *J Vector Borne Dis* 2011 Sep;48:180-1.

121. Gupta DK, Vaish AK, Arya RK, Chaudhary SC. Hypokalemic quadriparesis: an unusual manifestation of dengue fever. *BMJ Case Rep* 2011 Jun 17;2011.

122. Setiawan MW, Samsi TK, Wulur H, Sugianto D, Pool TN. Epigastric pain and sonographic assessment of the pancreas in Dengue hemmorhagic fever. *J Clin Ultrasound* 1998 Jun;26:257-9.

123. Karoli R, Fatima J, Singh G, Maini S. Acute pancreatitis: an unusual complication of dengue fever. *J Assoc Physicians India* 2012 Dec;60:64-5.

124. Srikiatkhachorn A, Krautrachue A, Ratanaprakarn W, Wongtapradit L, Nithipanya N, Kalayanarooj S. Natural history of plasma leakage in dengue hemorrhagic fever: a serial ultrasonographic study. *Pediatr Infect Dis J* Apr 2007;26:283-90;discussion 291-2.

125. Majumdar R, Jana CK, Ghosh S, Biswas U. Clinical spectrum of dengue fever in a tertiary care centre with particular reference to atypical presentation in the 2012 outbreak in Kolkata. *J Indian Med Assoc* 2012 Dec;110:904-6.

126. Sharma S, Sharma SK, Mohan A et al. Clinical profile of dengue hemorrhagic fever in adults during 1996 - Outbreak in Delhi, India. *Dengue Bull* 1998;22:20-7.

127. Sangle SA, Dasgupta A, Ratnalikar SD, Kulkarni RV. Dengue myositis and myocarditis. *Neurol India* 2010 Jul-Aug;58:598-9.

128. Wali JP, Biswas A, Chandra S, Malhotra A, Aggarwal P, Handa R, *et al*. Cardiac involvement in dengue hemorrhagic fever. *Int J Cardiol* 1998;64:31-6.

129. Carroll ID, Toovey S, Van Gompel A. Dengue fever and pregnancy- a review and comment. *Travel Med Infect Dis* 2007 May;5(3):183-8.

130. Perret C, Chanthavanich P, Pengsaa K: Dengue infection during pregnancy and transplacental antibody transfer in Thai mothers . *J Infect* 2005;51:287-93.

131. Sharma JB, Gulati N: Potential relationship between dengue fever and neural tube defects in a northern district of India. *Int J Gynaecol Obstet* 1992;39:291-5.

132. Sirinavin S, Nuntnarumit P, Supapannachart S, Boonkasidecha S, Techasaensiri C, Yoksarn S: Vertical dengue infection: case reports and review. *Pediatr Infect Dis J* 2004;23:1042-7.

133. WHO. Scientific Working Group Report on Dengue [online]<http://apps.who.int/tdr/publications/tdr-research-publications/swg-report-dengue/pdf/swg_dengue_2.pdf> (WHO, Geneva, Switzerland, 2007). p 93-105.

134. Halstead SB. Dengue. *Lancet* 2007 Nov 10;370:1644-52.

135. Alexander N, Balmaseda A, Coelho IC, Dimaano E, Hien TT, Hung NT, Jänisch T, Kroeger A, Lum LC, Martinez E, Siqueira JB, Thuy TT, Villalobos I, Villegas E,Wills B; European Union, World Health Organization (WHO-TDR) supported DENCO Study Group. Multicentre prospective study on Dengue classification in four South-east Asian and three Latin-American countries. *Trop Med Int Health* 2011 Aug;16:936-48.

136. Falconar AK, de Plata E, Romero-Vivas CM. Altered enzyme-linked immunosorbent assay immunoglobulin M (IgM)/IgG optical density ratios can correctly classify all primary or secondary dengue virus infections 1 day after the onset of symptoms, when all of the viruses can be isolated. *Clinical and Vaccine Immunology* 2006;13:1044–51.

137. Pelegrino JL. *Summary of dengue diagnostic methods*. World Health Organization, Special Programme for Research and Training in Tropical Diseases, 2006 (unpublished report).

138. WHO. Scientific Working Group Report on Dengue [online]<http://apps.who.int/tdr/publications/tdr-research-publications/swg-r

eport‐dengue/pdf/swg_dengue_2.pdf> (WHO, Geneva, Switzerland, 2007). p 28-40.

139. WHO. Scientific Working Group Report on Dengue [online]<http://apps.who.int/tdr/publications/tdr‐research‐publications/swg‐report‐dengue/pdf/swg_dengue_2.pdf> (WHO, Geneva, Switzerland, 2007). p 11.

140. Wiwanitkit V. Dengue fever: diagnosis and treatment. *Expert Rev Anti Infect Ther* 2010 Jul;8(7):841-5.

141. Damonte EB, Matulewicz MC, Cerezo AS. Sulfated seaweed polysaccharides as antiviral agents. *Curr. Med.Chem* 2004;11:2399-419.

142. Crance JM, Scaramozzino N, Jouan A, Garin D. Interferon, ribavirin, 6-azauridine and glycyrrhizin: antiviral compounds active against pathogenic flaviviruses. *Antiviral Res* 2003;58:73-9.

143. Yin Z, Chen YL, Schul W et al. An adenosine nucleoside inhibitor of dengue virus. *Proc. Natl Acad. Sci. USA* 2009;106:20435-9.

144. Kumar A, Rao CR, Pandit V, Shetty S, Bammigatti C, Samarasinghe CM. Clinical manifestations and trend of dengue cases admitted in a tertiary care hospital, Udupi, Karnataka. *Indian J Community Med* 2010 Jul;35:386-90.

145. Hati AK. Dengue serosurveillance in Kolkata, facing an epidemic in West Bengal, India. *J Vector Borne Dis* 2009 Sep;46:197-204.

146. Sanjay Kumar Mandal, Jacky Ganguly, Koelina Sil, Sumanta Chatterjee, Kaushik Chatterjee, Pankaj Sarkar et al. Clinical profile of dengue fever at a teaching hospital of Eastern India. *Nat. J Med Res* 2013 Apr-jun;3:173-6.

147. Murthy, J.M.K. Neurological complications of dengue infection. *Neurol. India* 2010 Oct-Dec;58:581-4.

ANNEXURE-I

ABBREVIATIONS:

DF- Dengue fever

DHF- Dengue Hemorrhagic fever

DSS- Dengue Shock syndrome

DENV- Dengue virus

ADE- Antibody dependent enhancement

EC- Endothelial call

TNFα- Tumor necrosis factor α

TGFβ- Transforming growth factor β

IFNγ- Interferon gamma

G6PD- Glucose 6 phosphate dehydrogenase

HLA- Human leucocyte antigen

IL- Interleukin

NO- nitric oxide

TCP- Thrombocytopenia

HCT- Hematocrit

AKI- Acute kidney injury

ALI/ARDS- Acute lung injury/ Acute respiratory distress syndrome

BUN- Blood urea nitrogen

SGOT/SGPT (AST/ALT)- Aspartate transaminase/ Alanine transaminase

INR- International normalised ratio

PT- Prothrombin time

aPTT- activated plasma thromboplastin time

PCR- Polymerase chain reaction

ANNEXURE II

Informed Consent Form (ICF) for Participants in Research Programmes involving studies on human beings

TITLE: -

Study Number: SVU/SBKS/ /201 -____

Participants Initials: _____

Participant's Name _____

Date of Birth / Age _____ (Years)

- I confirm that I have read and understood the information sheet dated _____ for the above study and have had the opportunity to ask questions.
- I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.
- I understand that the investigator of this study, others working on the investigator's behalf, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records, both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the study. I agree to this access. However, I understand that my identity will not be revealed in any information related to third party or published. I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s).
- I agree to take part in the above study.

Signature (or thumb impression) of the participant

Legally acceptable representative: _____

Signatory's Name:_____Date:_____

Signature of the investigator: _____

Date:_____

Study Investigator's Name:

Signature of the impartial witness: _____

Date:_____

Name of the witness: _____

પરિશિષ્ટ-૩

સુમનદીપવિદ્યાપીઠ

પીપરીયા. તા. વાઘોડિયા, જીલ્લો વડોદરા ૩૯૧૭૬૦

માનવીઓમાં થતા સંશોધન માટેનું ઇન્ફોર્મડ કન્સેન્ટ ફોર્મ

અભ્યાસનું શીર્ષક: -

અભ્યાસ નંબર: એસ.વી.યુ./એસ.બી.કે.એસ./ /૨૦૧૩ - _____

સહભાગીના પુરાનામનાપહેલાઅક્ષરો:_____

સહ્ભાગીનું નામ_____જન્મતારીખ/ ઉમર_____(વર્ષ)

- હું તારીખ _____ અને ઉપરના અભ્યાસનું માહિતીપત્રક વાંચી અને સમજીને અભ્યાસમાં સામેલ થવાની ખાતરી આપું છું અને મને કોઈપણ પ્રશ્નો પૂછવાની તક આપવામાં આવશે. જેની મને ખબર છે.
- મારી અભ્યાસમાં ભાગીદારી સ્વૈચ્છિક છે અને હું મારી ભાગીદારી કોઈપણ કારણ આપ્યા વગર, મારી તબીબી સારવાર ને અસરના પહોચે અથવા મારા કાનૂની અધિકારોને અસર ના થાય તેમ ગમે ત્યારે પાછી ખેચી લેવા સ્વતંત્ર છું.
- હું આ અભ્યાસના અભ્યાસકર્તા, અભ્યાસકર્તાના સહયોગી, એથીક્સકમિટી અને રેગ્યુલેટરી ઓથોરીટીસ ને મારી પરવાનગી વગર મારા સ્વાસ્થ્યનો રેકોર્ડ કે જે આ અભ્યાસ કે આભવિષ્યમાં થનારા પરીક્ષણના સંદર્ભમાં થશે તે ને જોવાની સંમતિ આપું છું. જો હું મારી સંમતિ પછી ખેચી લઉં તો પણ મારી માહિતી અભ્યાસકર્તા

મેળવી શકશે.છતાં, હું સમજુ છું કે, મારી ઓળખાણ કોઈપણ સ્વરૂપમાં ત્રીજી વ્યક્તિને અપાશે નહી કે જાહેરમાં પ્રકાશિત કરવામાં નહિ આવે.

- આ અભ્યાસ પરથી જે પરિણામ કે માહિતી મળે તેની પર હું પ્રતિબંધ નહિ મુકું. તેનો ઉપયોગ ફક્ત વૈજ્ઞાનિક હેતુ માટે કરવામાં આવશે.
- હું ઉપરના અભ્યાસમાં ભાગ લેવાની સંમતિ આપું છું.
- સહી અથવા અંગુઠાનું નિશાન_____

અથવા

કાયદાકીય રીતે સ્વીકાર્ય પ્રતિનિધિ_____

સહીકર્તાનું નામ_____તારીખ_____

અભ્યાસકર્તાની સહી_____તારીખ_____

અભ્યાસના અભ્યાસકર્તાનું નામ_____

નિષ્પક્ષસાક્ષીની સહી_____તારીખ_____

નિષ્પક્ષસાક્ષીનું નામ_____

ANNEXURE IV

PATIENT INFORMATION SHEET

STUDY TITLE : "**A STUDY OF CLINICO-HAEMATOLOGICAL PROFILE OF PATIENTS WITH DENGUE FEVER**

1. INTRODUCTION

   Dengue Fever is an infection that has been prevalent in India for more than two centuries Epidemic Dengue Fever and Dengue Hemorrhagic fever have emerged as a global public health problem in recent decades. In fact, the problem has become hyperendemic in many urban and rular areas, with frequent epidemics.

2. What is the purpose of the study?

   For Post Graduation Research Work, Essential for examination criteria.

3. Why have I been chosen ?

   Because You are Suitable participant according to the need of the study

4. Do I have to take part ?

   The study is absolutely voluntary in nature. Participants can participate in this study willingly after understanding about the study

5. How long will the study last ?

   The study will last for two years

6. What will happen to me if I take part?

   There are no expected adverse events or risk expected because it is an observational study of investigation.

7. What I have to do?

   We need your consent for this study. No extra efforts are required from your side.

8. What is the drug being tested?

   No drug is being tested

9. What are the benefits of the study ?

   This study helps in early and better diagnosis. It will lead to effective treatment and better prognosis.

10. What are the alternative for the treatment ?

    Not applicable

11. What if new information becomes available?

    If new information becomes available , it will be included in management process. It is not going to affect the study.

12. What happens when study stops?

    All the data will be analysed and compiled in the form of a Dissertation and submitted to the university.

13. What if something goes wrong?

    The principle investigator will take care during the course of the study if something goes wrong

14. Will my taking part be kept confidential?

    During the study, all data collected will be confidential. Your privacy will be maintained.

15. What else should I know?

    No extra expense will be borne by the patient related to the study. Just by their participation and little contribution, the clinic haematological profile of Dengue will be better understood.

16. Additional precautions

    Nothing at this point of time.

17. Who to call with question?

    Dr Varun Desai, Department Of Medicine Dhiraj General Hospital, Pipariya - 391760 Waghodia, Vadodara , Gujarat

ANNEXURE V

દરદી માહિતી શીટ

અભ્યાસ શીર્ષક: અભ્યાસનું ટાઇટલ: "ડેંગ્ગ ફેવર સાથે દર્દીઓના ક્લિનકો-હેમોટોોલોજિક પ્રોફાઇલનો અભ્યાસ

1. પરિચય

   ડેન્ગ્યુ ફીવર એક ચેપ છે જે ભારતમાં બેથી વધુ સદી માટે પ્રચલિત છે તાજેતરના દાયકામાં રોગચાળો ડેન્ગ્યુ ફીવર અને ડેન્ગ્યુ હેમોરેજિક તાવ વૈશ્વિક જાહેર આરોગ્ય સમસ્યા તરીકે ઉભરી આવ્યો છે. હકીકતમાં, ઘણી શહેરી અને રુધિર વિસ્તારોમાં આ સમસ્યા હાઇપ્રેન્ડેમિક બની ગઇ છે, જેમાં વારંવાર રોગચાળો આવે છે.

2. અભ્યાસનો હેતુ શું છે?

   પોસ્ટ ગ્રેજ્યુએશન રીસર્ચ વર્ક માટે, પરીક્ષા માપદંડ માટે આવશ્યક છે.

3. મને શા માટે પસંદ કરવામાં આવ્યો છે?

   કારણ કે તમે અભ્યાસની જરૂરિયાત અનુસાર યોગ્ય સહભાગી છો

4. શું મને ભાગ લેવાની જરૂર છે?

   અભ્યાસ સંપૂર્ણપણે પ્રકૃતિ સ્વૈચ્છિક છે. અભ્યાસ વિશે સમજ્યા પછી સહભાગીઓ સ્વેચ્છાએ આ અભ્યાસમાં ભાગ લઇ શકે છે

5. કેટલા સમય સુધી અભ્યાસ ચાલશે?

   અભ્યાસ બે વર્ષ સુધી ચાલશે

6. જો હું ભાગ લેતો હોઉં તો શું થશે?

   અપેક્ષિત પ્રતિકૂળ ઘટનાઓ અથવા જોખમ અપેક્ષિત છે કારણ કે તે તપાસનું નિરીક્ષણક અભ્યાસ છે

7. મારે શું કરવું છે?

   આ અભ્યાસ માટે અમને તમારી સંમતિની જરૂર છે તમારી બાજુએ કોઇ વધારાની પ્રયત્નોની આવશ્યકતા નથી.

8. ડ્રગ કેવી રીતે પરીક્ષણ કરવામાં આવે છે?

   કોઇ દવા પરીક્ષણ કરવામાં આવી રહી છે

9. અભ્યાસના લાભો શું છે?

   આ અભ્યાસ પ્રારંભિક અને બહેતર નિદાનમાં મદદ કરે છે. તે અસરકારક ઉપચાર અને સારા પૂર્વસૂચન તરફ દોરી જશે.

10. સારવાર માટે વૈકલ્પિક શું છે?

    લાગુ નથી

11. જો નવી માહિતી ઉપલબ્ધ થાય તો શું?

    જો નવી માહિતી ઉપલબ્ધ બને, તો તેને મેનેજમેન્ટ પ્રક્રિયામાં શામેલ કરવામાં આવશે. તે અભ્યાસ પર અસર નથી જઈ રહ્યા છે.

12. જ્યારે અભ્યાસ બંધ થાય ત્યારે શું થાય?

    બધા ડેટા વિશ્લેષણ કરવામાં આવશે અને ડીઝર્ટેશનના સ્વરૂપમાં સંકલિત થશે અને યુનિવર્સિટીને સુપરત કરવામાં આવશે.

13. જો કંઈક ખોટું થાય તો શું?

    જો કંઈક ખોટું થાય તો અભ્યાસના અભ્યાસ દરમિયાન સિધ્ધાંત તપાસકાર કાળજી લેશે

14. શું મારો ભાગ લેવાથી ખાનગી રાખવામાં આવશે?

    અભ્યાસ દરમિયાન, એકત્રિત કરવામાં આવેલી તમામ માહિતી ગુપ્ત રહેશે. તમારી ગોપનીયતા જાળવવામાં આવશે

15. મને બીજું શું જાણવું જોઈએ?

    કોઈ વધારાની ખર્ચ અભ્યાસ સાથે સંબંધિત દર્દી દ્વારા જન્મે આવશે. માત્ર તેમની સહભાગિતા અને બહુ ઓછું યોગદાન દ્વારા, ડેન્ગ્યુના ક્લિનિક હીમોટિકલ પ્રોફાઇલને વધુ સારી રીતે સમજી શકાય છે.

16. વધારાની સાવચેતીઓ

    આ બિંદુ પર કંઈ નથી

17. પ્રશ્ન સાથે કૉલ કરવા માટે કોણ ?

    ડૉ. વરુણ દેસાઇ, ધીરજ જનરલ હોસ્પિટલ, પિપરિયા - 391760

    વાઘોડિયા, વડોદરા, ગુજરાત

ANNEXURE VI

रोगी सूचना पत्र

अध्ययन शीर्षक: "डेंगू फारे के साथ रोगियों के क्लिनो-हेमेटोलॉजिकल शख्सियत का एक अध्ययन

1। परिचय
डेंगू बुखार एक संक्रमण है जो भारत में दो से ज्यादा शतकों के लिए प्रचलित है हाल के दशकों में महामारी डेंगू बुखार और डेंगू हार्म्राजिक फिवर वैश्विक सार्वजनिक स्वास्थ्य समस्या के रूप में उभरा है। वास्तव में, समस्या कई शहरी और राउलर क्षेत्रों में लगातार अति महामारियों के साथ अतिसंवेदनशील बन गई है।

2. अध्ययन का उद्देश्य क्या है?
परीक्षा स्नातक अनुसंधान कार्य के लिए, परीक्षा मानदंडों के लिए आवश्यक

3. क्यों मुझे चुना गया है?
क्योंकि आप अध्ययन की आवश्यकता के अनुसार उपयुक्त भागीदार हैं

4. क्या मुझे भाग लेना है?
अध्ययन पूरी तरह स्वैच्छिक है अध्ययन के बारे में समझने के बाद प्रतिभागी स्वेच्छा से इस अध्ययन में भाग ले सकते हैं

5. अध्ययन कब तक खत्म होगा?
अध्ययन दो साल तक चलेगा

6. अगर मैं भाग लेता हूं तो मेरे साथ क्या होगा?
कोई अपेक्षाकृत प्रतिकूल घटनाएं या जोखिम की संभावना नहीं है क्योंकि यह जांच का एक अवलोकन अध्ययन है

7. मुझे क्या करना है?
हमें इस अध्ययन के लिए आपकी सहमति की आवश्यकता है। आपके पक्ष से कोई अतिरिक्त प्रयास की आवश्यकता नहीं है

8. क्या दवा का परीक्षण किया जा रहा है?
कोई दवा का परीक्षण नहीं किया जा रहा है

9. अध्ययन के क्या लाभ हैं?
यह अध्ययन जल्दी और बेहतर निदान में मदद करता है। इससे प्रभावी उपचार और बेहतर रोग का निदान होगा।

10. उपचार के लिए वैकल्पिक क्या हैं?
लागू नहीं

11. यदि नई जानकारी उपलब्ध हो तो क्या?
यदि नई जानकारी उपलब्ध हो जाती है, तो इसे प्रबंधन प्रक्रिया में शामिल किया जाएगा। यह अध्ययन को प्रभावित करने वाला नहीं है।

12. क्या होता है जब अध्ययन बंद हो जाता है?
सभी डेटा का विश्लेषण किया जाएगा और एक निबिंध के रूप में संकलित किया जाएगा और विश्वविद्यालय को प्रस्तुत किया जाएगा।

13. अगर कुछ गलत हो जाए तो क्या होगा?
यदि कुछ गलत हो जाता है तो अध्ययन के दौरान सिद्धांत अन्वेषक ध्यान में रखेगा

14. क्या मेरा भाग लेना गोपनीय रखा जाएगा?
अध्ययन के दौरान, एकत्र किए गए सभी डेटा गोपनीय होंगे। आपकी गोपनीयता बनाए रखा जाएगा

15. मुझे और क्या पता होना चाहिए?
अध्ययन से संबंधित रोगी द्वारा अतिरिक्त खर्च नहीं किया जाएगा। सिर्फ उनकी भागीदारी और थोड़ा योगदान से, डेंगू का क्लिनिकि हीमॅटोलॉजिकिल प्रोफाइल बेहतर होगा।

16. अतिरिक्त सावधानी
इस समय के कुछ भी नहीं।

17. प्रश्न के साथ कॉल करने के लिए
डॉ। वरुण देसाई, चिकित्सा विभाग धीरज जनरल अस्पताल, पीपरिया - 391760
वाघोडिया, वडोदरा, गुजरात

PROFORMA

NAME AGE/SEX OPD/IPD

CLINCAL PROFILE

FEVER

MYALGIA

HEADACHE

RETRO-ORBITAL PAIN

NAUSEA

VOMITING

BLEEDING MENIFESTATIONS

RASH

ABDOMINAL PAIN

HEPATOSPLENOMEGALY

BREATHLESSNESS

DIARRHOEA

OLIGURIA

JAUNDICE

ASCITES

HYPOTENSION

ALTERED SENSORIUM

CONVULSIONS

UNCONSCIOUSNESS/COMA

HEMATOLOGIC AND URINE PROFILE

HB

TC

DC

PLT

HEMATOCRIT

PT

APTT

URINE

DENGUE PROFILE

NS1

IgG

IgM

BIOCHEMICAL PROFILE

RBS

Na

K

UREA

CREAT

SGPT

SGOT

BILIRUBIN

TOTAL

DIRECT

ALBUMIN

RADIOLOGI PROFILE

CHEST XRAY

PLEURAL EFFUSION

USG ABDOMEN

HEPATOMEGALY

SPLENOMEGALY

CHOLESTASIS

OTHERS

DIAGNOSIS

DF

DHF

DSS

MASTER CHART

SR NO.	AGE	SEX	FEVER	MYALGIA	HEADACHE	RETRO-ORBITAL PAIN	NAUSEA	VOMITING	TOURNIQUET TEST POSITIVE	RASH	EPISTAXIS	HEMATURIA	ABDOMINAL PAIN	BREATHLESSNESS	DIARRHOEA	ALTERED SENSORIUM	OLIGURIA	JAUNDICE	CONVULSIONS	PLEURAL EFFUSION	ASCITED	HEPATOMEGALY	SPLENOMEGALY	HYPOTENSION(SBP<90)	HEMOGLOBIN	TOTAL COUNTS	DC	PLATELETS	HEMOCRIT	PT	APTT	RBS	Na+	K+	UREA	CREATININE	SGPT	SGOT	TOTAL BILIRUBIN	DIRECT BILIRUBIN	TOTAL PROTEIN	S. ALBUMIN	NS1	IgM	IgG	DIAGNOSIS
1	25	F	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	12	4600	60/32/4/4	80000	40	13.8	38	120	130	4	17	1.2	27	26	0.9	0.3	7	3.2	YES	NO	NO	DF
2	33	M	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	14	4200	76/25/4/5	90000	36	13.8	38	89	126	3.8	32	0.7	44	48	0.7	0.3	7.2	3.2	YES	NO	NO	DF
3	43	F	YES	YES	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	8.8	5000	63/28/4/5	120000	29.7	13.8	38	100	138	4.6	25	1.1	113	270	0.8	0.3	6	3	YES	NO	NO	DF
4	35	F	YES	YES	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	13	12000	60/31/4/5	120000	38.1	13.8	38	90	138	4.2	27	1.5	40	44	0.9	0.4	6.3	3	YES	NO	NO	DF
5	30	M	YES	YES	YES	YES	YES	YES	YES	YES	NO	YES	YES	YES	NO	NO	YES	YES	NO	YES	YES	YES	YED	YES	14	4300	66/25/4/5	40000	52.2	22	45	116	135	3.9	21	0.7	177	291	1.3	0.6	5	2.3	YES	NO	NO	DSS
6	35	M	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	11	5600	70/22/4/4	80000	34	13.8	38	76	136	4.3	17	0.5	27	17	0.7	0.3	6	2.8	NO	YES	NO	DF
7	19	M	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	13	3000	50/41/4/5	110000	39.1	13.8	38	92	131	3.4	42	1.1	38	66	0.5	0.2	6.3	3.3	NO	YES	NO	DF
8	19	M	YES	YES	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	14	2500	60/31/4/5	80000	41.9	13.8	38	86	136	4.8	34	1.5	35	124	0.6	0.3	6.8	3.4	YES	NO	NO	DF
9	35	M	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	15	10000	41/50/4/5	40000	45.6	13.8	40.2	121	140	4.2	37	1	22	27	0.8	0.3	7.5	2.4	YES	NO	NO	DF
10	50	M	YES	YES	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	14	3200	60/31/4/5	70000	46.2	13.8	41	155	135	3.4	27	1.2	29	47	0.4	0.2	7	3.6	NO	YES	YES	DF
11	23	F	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	YES	YES	NO	15	11800	74/18/4/4	20000	54.1	16.3	38	136	133	4.2	23	0.7	57	199	1	0.4	5	2.7	YES	NO	NO	DHF
12	22	M	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	YES	NO	YES	YES	NO	14	3500	50/41/4/5	40000	51	15	42	129	138	3.4	13	0.8	34	41	0.6	0.3	7.4	3	YES	NO	NO	DHF
13	18	M	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	13	8100	77/14/4/5	180000	40.5	13.8	38	111	139	3.7	27	0.7	24	27	0.8	0.3	7	3.2	YES	NO	NO	DF
14	50	F	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	11	7000	54/41/2/3	110000	33.1	15.1	38	123	137	3.6	32	0.9	57	71	0.8	0.4	7.1	3.4	NO	YES	YES	DF
15	30	F	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	NO	NO	YES	YES	NO	YES	YES	YES	YES	YES	7.1	3000	55/36/4/5	67000	39	16.3	59.5	134	126	4.4	122	4	200	206	9.7	6	5.2	2.3	NO	YES	NO	DSS
16	35	M	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	13	5700	71/20/4/5	150000	38.8	13.8	38	105	136	4	21	0.8	16	32	0.8	0.4	6.8	3.5	YES	NO	NO	DF
17	32	M	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	15	4000	49/42/4/5	80000	40.9	13.8	38	106	133	4	18	0.8	41	87	0.6	0.3	6.5	3.5	YES	NO	NO	DF
18	23	M	YES	YES	YES	YES	YES	YES	YES	YES	NO	YES	YES	NO	NO	NO	NO	NO	NO	YES	NO	YES	YES	NO	14	7200	49/40/6/5	30000	39.6	13.8	38	131	136	4.1	36	1.2	413	656	1.2	0.5	6.4	3	NO	NO	YES	DHF
19	23	F	YES	YES	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	12	4800	49/42/4/5	70000	36.8	13.8	38	91	137	4.3	20	0.7	17	14	0.7	0.3	6.2	3	YES	YES	YES	DF
20	22	F	YES	YES	YES	YES	YES	YES	YES	YES	NO	YES	YES	NO	NO	NO	NO	YES	NO	YES	NO	YES	YES	NO	7.2	3000	51/40/4/5	1,20,000	21.2	15	38	76	135	4.4	16	0.6	56	121	1.7	0.6	6.3	3.3	YES	YES	YES	DHF
21	35	F	YES	YES	YES	YES	YES	YES	YES	YES	NO	YES	YES	YES	NO	YES	YES	YES	NO	YES	YES	YES	YES	YES	9.9	3480	61/30/4/5	50000	38	35	38	77	128	3.9	91	1.5	112	229	9.3	5.5	5.7	2.2	NO	YES	NO	DSS
22	45	M	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	13	10300	74/20/3/3	140000	37.2	13.8	38	109	130	3.6	29	1.4	66	87	0.7	0.3	6.8	3.2	NO	YES	NO	DF
23	34	F	YES	YES	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	11	3000	46/45/4/5	100000	32	13.8	38	74	132	4.1	19	0.7	21	20	0.8	0.3	6.4	3.4	YES	NO	NO	DF
24	70	M	YES	YES	YES	YES	YES	YES	YES	YES	NO	YES	YES	YES	NO	YES	YES	YES	NO	YES	NO	YES	YES	NO	8.9	15500	81/10/4/5	10000	24.5	13.8	46	124	141	3.2	103	1.8	90	106	2.5	1.5	5.4	2.4	NO	YES	NO	DHF
25	22	M	YES	YES	YES	YES	YES	YES	YES	YES	NO	YES	NO	NO	NO	NO	NO	NO	NO	YES	NO	YES	YES	NO	13	2000	62/26/6/6	50000	35.6	17.6	37.2	126	130	3.4	35	1	56	132	0.6	0.6	6.2	3.4	YES	NO	NO	DHF
26	20	M	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	16	3100	44/47/4/5	100000	45.8	13.8	41	85	128	4.5	29	1.2	40	34	0.9	0.3	6.8	3.4	YES	NO	NO	DF
27	45	M	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	11	4000	55/36/4/5	120000	34.6	13.8	42	116	141	4.9	23	0.7	12	34	0.3	0.1	6.9	3.5	YES	NO	NO	DF
28	31	F	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	14	4000	63/28/4/5	70000	39	15.2	42	102	137	4.7	15	0.6	30	75	0.4	0.2	6.8	3.6	YES	NO	NO	DF
29	22	F	YES	YES	YES	YES	YES	YES	NO	NO	NO	NO	YES	NO	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	13	2500	60/29/5/6	80000	37.9	13.8	38	104	138	3.5	14	0.7	66	74	0.3	0.2	6.4	3.4	YES	NO	NO	DF
30	35	F	YES	YES	YES	YES	YES	YES	YES	YES	NO	YES	YES	YES	YES	YES	YES	YES	NO	YES	YES	YES	YES	YES	11	3600	45/46/5/4	40000	41	18	55	71	126	2.9	42	2	102	146	4.4	2.5	5	2.6	NO	YES	NO	DSS
31	35	F	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	11	4000	50/41/4/5	80000	32	15	42	81	138	5	27	0.7	27	14	0.8	0.3	6.8	3.6	NO	YES	NO	DF
32	27	F	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	YES	NO	YES	YES	NO	7.6	10300	81/11/4/4	140000	23.6	20	50	90	130	3	120	2.3	56	59	2	1.4	5.2	2.9	YES	NO	NO	DHF
33	55	F	YES	YES	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	13	5700	50/38/2/10	70000	40.1	15	38.9	129	130	3.6	27	1.1	34	37	0.9	0.2	5.8	2.7	YES	YES	YES	DF
34	18	F	YES	YES	YES	YES	YES	YES	NO	NO	NO	NO	YES	NO	YES	NO	NO	NO	NO	NO	NO	YES	YES	NO	8.3	7000	71/20/4/5	110000	29	20	44	96	126	3.5	26	1.1	27	26	0.6	0.2	6.8	3.2	YES	NO	NO	DF
35	46	M	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	15	4000	60/32/4/4	40000	44	13.8	38.8	79	135	3.9	20	1	21	20	0.8	0.2	6.8	3.2	YES	NO	NO	DF
36	39	F	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	11	3000	50/40/5/5	80000	32	13.8	38	94	126	3.6	21	0.7	42	50	1	0.3	6.9	3.4	YES	NO	NO	DF
37	20	M	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	YES	YES	NO	14	3500	66/28/3/3	160000	40.8	13.8	38	120	139	4.5	30	1.5	36	30	1.2	0.4	6.8	3.6	YES	NO	NO	DF
38	45	F	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	12	2800	50/45/2/3	80000	33	13.8	38	109	136	4.2	28	0.7	17	11	0.4	0.2	6.8	3	YES	NO	NO	DF
39	18	M	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	14	4000	58/33/4/5	60000	41.5	13.8	38	86	139	4.5	29	1.2	26	22	1.2	0.5	6.9	3.6	YES	NO	NO	DF
40	18	M	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	YES	YES	NO	14	4200	45/50/2/3	60000	41	13.8	38	79	146	4.5	29	1.4	37	32	0.9	0.2	6.8	3.4	YES	NO	NO	DF
41	41	F	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	YES	YES	NO	12	4500	445/45/6/4	60000	35	13.8	38	91	139	4.6	29	1.1	27	20	1.1	0.4	6.9	3.1	YES	NO	NO	DF
42	18	M	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	10	12000	71/20/4/5	439000	29.6	13.8	38	88	140	4.2	59	2.4	25	25	0.6	0.2	7.2	4	NO	YES	NO	DF
43	28	F	YES	YES	YES	YES	YES	YES	NO	NO	NO	NO	YES	NO	NO	NO	NO	NO	NO	NO	NO	YES	YES	NO	10	3500	50/42/4/4	80000	31.7	13.8	38	81	142	4.3	19	0.7	34	49	0.5	0.2	7	3.8	YES	NO	NO	DF
44	32	F	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	YES	NO	NO	NO	YES	YES	NO	12	10200	61/31/4/4	285000	37.8	13.8	41	87	142	4.6	80	1.1	44	46	2	1.1	6.9	3.4	YES	NO	NO	DF
45	41	M	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	14	15000	54/38/4/4	150000	42.1	15	38	95	136	3.7	30	1.4	138	114	1	0.4	7.2	3.6	YES	YES	NO	DF
46	26	M	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	11	4200	63/28/4/5	120000	37.6	13.8	38	82	138	3.8	29	1.5	38	52	0.8	0.3	7.1	4	NO	YES	NO	DF
47	53	M	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	14	3300	45/44/6/5	70000	41.2	13.8	38	79	142	4.6	26	1.1	112	114	1.2	0.5	7.1	3.6	YES	YES	YES	DF
48	26	M	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	14	4000	50/40/4/6	40000	42.3	13.8	38	95	142	4.8	26	1	47	44	1.2	0.4	7.2	4	NO	YES	NO	DF
49	45	M	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	YES	YES	NO	14	5200	46/46/4/4	30000	44.2	13.8	38	81	146	4.2	29	1.5	37	45	1.5	0.6	7.5	3.5	YES	NO	NO	DF
50	55	M	YES	YES	YES	YES	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	16	5000	42/50/4/4	90000	47.6	13.8	38	101	143	3.3	37	1.5	57	56	1.1	0.3	7	3.6	YES	NO	NO	DF