DENGUE: A CLINICOHAEMATOLOGICAL PROFILE AND ROLE OF PLATELET TRANSFUSION IN ITS MANAGEMENT

Dissertation submitted in partial fulfillment of the requirements for the degree of

M.D. (PATHOLOGY)
Branch III

APRIL- 2015

THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY

CHENNAI – TAMILNADU

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CERTIFICATE

This is to certify that the dissertation entitled **"DENGUE-A CLINICOHAEMATOLOGICAL PROFILE AND ROLE OF PLATELET TRANSFUSION IN ITS MANAGEMENT"** is a bonafide work done by **Dr.EVELYN ANGEL.S.** in the **DEPARTMENT OF PATHOLOGY,SREE MOOKAMBIKA INSTITUTE OF MEDICAL SCIENCES,KULASEKHARAM** in partial fulfillment of the University rules and regulations for award of **M.D DEGREE IN PATHOLOGY** under my guidance and supervision during the academic year 2012-2015.

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"Excellence measures a man by the height of his ideals, the depth of his convictions,the length of his persistence and the breadth of his compassion"

CONTENTS

ABSTRACT

Aim: Dengue is a major health problem and ranks as the most important mosquito borne viral disease in the world.Several factors have been attributed to increased morbidity and mortality in dengue with altered haematological and coagulation parameters playing an important role.The present study was done to analyze the haematological findings and correlate the same with the clinical course ,to evaluate the role of platelet count in predicting outcome in dengue patients and to observe the number of serologically dengue positive patients requiring platelet transfusion and its outcome.

Materials and Methods: Fifty patients admitted in Sree Mookambika Institute of Medical Sciences Hospital with fever and IgM and or NS1 positivity were selected.Various haematological,biochemical and clinical parameters were studied along with the role of platelet transfusion and the outcome noted.Statistical analysis was done for the various parameters.

Results: Out of the 50 cases in this study all the cases had fever(100%),headache (100%),myalgia (86%) ,hepatomegaly (52%) and splenomegaly in (48%).26% had ascites,rise of haematocrit and pleural effusion was seen in 16% each.Leukopenia was seen in 73% of the cases ,reversal of neutrophil lymphocyte ratio was seen in 27% of the cases. AST and ALT levels were raised with mean value being 106.08 IU/L and 118.54 IU/L.Platelet transfusion was indicated 86% of the cases.52% of the cases were transfused platelets on the first day.54%of the cases had 1-2 units of platelets transfused. 53.49% of the patients took 3 days to recover 3 days after transfusion of platelets.

Conclusion: Fever with headache and presence of thrombocytopenia,leukopenia,reversal of neutrophil lymphocyte ratio with signs of capillary leak like ascitis ,pleural effusion and rising haematocrit should raise strong suspicion of dengue fever.Platelet transfusion in cases of moderate to severe thrombocytopenia proved to be useful for an eventful and smooth recovery.

Key words: Dengue,IgM,Thrombocytopenia,Leukopenia,reversal of neutrophil lymphocyte ratio, rising haematocrit,Platelet transfusion

"*The pains which accompanied this fever were exquisitely severe in the head, back and limbs. The pains in the head were sometimes in the back parts of it, and at other times they occupied only the eyeballs. In some people the pains were so acute in their backs and hips that they could not lie in bed… A few complained of their flesh being sore to touch, in every part of the body. From these circumstances, the disease was sometimes believed to be rheumatism. But its more general name among all classes of people was Break – Bone fever*."

Benjamin Rush (1745-1813)

The name Dengue has an African origin. The term 'dengue' is a Spanish adaptation at the swahill expression*'Ki-Dinga pepo'* meaning 'cramp like seizures caused by evil spirits' reported during 19th century. Epidemics of illness compatible with dengue fever were first reported in medical literature in 1779 in Butavia (present day Jakarta). It was also reported in 1780 in Philadelphia.

The Dengue virus was first isolated in the year 1943 and Dengue fever (DF) has been recognized for at least several hundred years.

The first reported epidemics of dengue illness occurred in 1779-1780 in Asia, Africa and North America. The near concurrent incidence of outbreak in three continents shows that these viruses and their mosquito vectors have had a world wide circulation in the tropics for more than two hundred years. For the duration of this time DF was considered a placid, nonfatal disease of visitors to the tropics. Usually, there were long intervals of 10-40 years between major epidemics chiefly because the beginning of a new serotype in a at risk population occurred only if viruses and their mosquito vector could endure the

slow transportation. A pandemic of dengue began in South East Asia after World War II and has spread around the world. Since then Epidemics caused by multiple serotypes (Hyperendemictity) are more recurrent, the geographic circulation of dengue viruses and their mosquito vectors has extended and DHF has emerged in the Americas and the Pacific region.

The worldwide epidemiology of dengue fever / dengue haemorrhagic fever is changing fast. The Indian encounter with this disease is fascinating and captivating. In recent years the disease has distorted its course manifesting in the severe form as DHF and with increasing incidence of outbreaks.

The reasons for this revival are:

1. Unparalleled growth of human populace.
2. Unintended and unrestrained inhabitation
3. Insufficient waste disposal and water supply
4. Amplified circulation & density of vector mosquitoes.
5. Lack of effective mosquito control procedures
6. Increased progression and spread of dengue virus.

Haematological parameters like Hb level, total WBC count, differential WBC count and platelet count are altered in dengue fever. The most common findings are thrombocytopenia with concurrent haemoconcentration.

A guide for analysis, treatment and containment of Dengue fever prepared by Technical Advisory Committee on Dengue Haemorrhagic fever for South East Asia Western Pacific areas in 1975 & 1980 has been modified by WHO.The criteria include 4 Major manifestations such as:

I. Major manifestations of fever

II. Haemorrhagic manifestations

III. Hepatomegaly

IV. Tendency to develop shock, two laboratory changes (ie) thrombocytopenia and concurrent haemoconcentration which have been proven to be practical for suspicion and subsequent screening in 95% cases.

Other important laboratory findings:

1. Leucopenia
2. Lymphocytosis with reactive Lymphocytes
3. Increased capillary permeability
4. Elevated liver enzymes

Aberrant immune overactivation as well as dengue virus induced toxicity contributes to disease process. Aberrant immune overactivation not only impairs clearance of virus but also results in:

1) Overproduction of cytokines like TNFα,TNF β,TNFγ that affect monocytes, endothelial cell and hepatocyte functions.
2) Abnormal overproduction of auto antibodies to platelets and endothelial cells through molecular mimicry to NS1, and prM antigens.

Dengue virus induced coagulopathy and vasculopathy and dengue virus associated inhibition of Megakaryopoiesis and apoptotic death of early megakayocytes progenitors also contribute to the pathogenesis.

An isolated platelet count of <50,000/µl is associated with minor risk of bleeding until the count drops below <1000-20,000/µl. There is increased risk of bleeding when associated with coexisting coagulopathies, liver disease,

platelet inhibiting drugs and infection. The minimum threshold for platelet transfusion is <10,000/µl in otherwise stable patients.

Today Dengue has positioned itself as the most important mosquito borne viral disease in the world. Current estimates report that at least 112 countries are endemic for Dengue and around 40% of the world population are at risk in tropics and subtropics. Annually around 100 million cases of dengue fever and half a million cases of DHF occurs world wide. Early recognition and prompt initiation of treatment and platelet transfusion in indicated cases are imperative if disease related morbidity and mortality are to be restricted.

1. To analyze the hematological findings in dengue illness and to correlate the same with the clinical course.
2. To evaluate the role of platelet count in predicting outcome in dengue patients.
3. To observe the number of serologically dengue positive patients requiring platelet transfusion and observe its outcome

History:

The word “Dengue”has an African origin[1,2,7]. The term "break-bone fever" was coined by Benjamin Rush who described dengue during an epidemic occurring in Philadelphia in 1780. Dengue epidemics are acknowledged to have occurred over the last three centuries in humid, subtropical and temperate places around the earth. A disease epidemic attuned with dengue was reported in China as early on as 992 AD, but the first outbreak of dengue was recorded in 1635 in the French West Indies.[1]

The earliest reported scourge of DF occurred between 1779- 1780 in Asia, Africa and North America. During initial epidemics, dengue was thought of as a mild, non fatal disease and there were extended intervals between the major outbreaks.[1]

A pandemic of DF started in South East Asia subsequent to World War II and has extended around the world since then. In these regions epidemics of DHF started in emerge in the 1950's, but by 1975 had become a major reason of hospitalization and death.[1,2]

Spread of dengue is now seen in every World Health Organisation (WHO) region of the world and more than 125 countries are recognized to be dengue endemic. The exact impact of dengue internationally is difficult to establish due to factors such as poor disease scrutiny, misdiagnosis and low level of coverage. Currently available data grossly underestimates the societal, monetary and illness burden[2]. Every ten years the usual yearly number of cases of DF/ DHF reported to WHO continues to raise exponentially.[1]From 2000 to 2008, the typical yearly number of cases was 1,656,870 (or) nearly thrice the

figure from 1990 – 1999, which was 4,79,848 of cases. In the year 2008, a record of 69 nations from the WHO regions of South –East Asia, Western Pacific and the Americas had dengue.[1,2]A 2012 publication recommended that 2.97 people living in 128 countries are at risk of dengue internationally, 834 million people in metropolitan residences and 763 million per cities. The same assemblage again in 2013 using cartographic approach said that 390 million dengue infections occur world wide.[2,6]

All the four strains of dengue viruses are circulating in continents of Asia, Africa and the Americas. Owing to early recognition and better case management, reported case casualty rates have been lower in the current years than in the decades before 2000.

The burden of infirmity caused by dengue is calculated by a set of epidemiological indicators such as the number of clinical cases classified by relentlessness of the disease (DF, DHF, DSS), duration of sickness period, value of life during the illness event, fatal outcome rate and total number of deaths during a given time frame. All these epidemiological indications are united into a single health indicator, such as disability – adjusted life years (DALYS).[1]

The South East Asia Region :

Of the 25 billion citizens around the globe existing in dengue endemic nations and at risk of having DF/ DHF, 1.3 billion live in nations of the WHO South East Asia (SEA) region which are known for being dengue endemic areas. Epidemics continue to persist at expected 3-5 years cycles all over SEA.

Till the year 2003, only eight nations in the area had reported dengue outbreak cases. By 2009, all countries except Korea had reported dengue outbreaks.[1]

The accounted for dengue cases and deaths from 1985 and 2009 in 10 countries of the WHO- SEA nations highlight the public health magnitude of this disease in the region[1].

The figure of dengue cases has amplified over the last three to four years with frequent epidemics[1]. Severe dengue is widespread in most SEA countries, with rates of severe dengue being 18 times high in this area compared with the Americas.[2]

In nations of the SEA region the tendency of dengue cases is showing an augment over the years. The case fatality rate (CFR), has however shown a waning tendency since 1985 and this is found to be accredited to better case management.[1]

The Indian Scenario :

The earliest epidemic of clinically suspicious dengue like fever was recorded at Chennai in 1780 and the first virologically proved outbreak of DF in India happened in Calcutta and the Eastern Coast of India in the years 1963-1964. The initial chief outbreak of DF occurred in 1953- 1954 in Philippines followed by a rapid universal increase of epidemics of DHF/ DF. DHF was occurring in the neighboring countries but it was not present in India for mysterious reasons as all risk factors were present.[6,8]

The DHF had started emerging in different parts of India ever since 1988.[1,4] The first major extensive epidemics of DHF/ DSS occurred in India in

the year 1996 in areas around Delhi and Lucknow and then it widened to all over the country.[4,8]

The epidemiology of dengue illness in the Indian region has been very multifaceted and has considerably changed over approximately past six decades in conditions of prevalent strains, affected geographical setting and ruthlessness of the disease.[4] The outbreak at Kanpur during the year 1968 was because of DV-4 and during the 1969 epidemic both DV-4 and DV-2 strains were isolated.[4] It was entirely replaced by the DV-2 Strain in 1970 outbreak in the adjoining city of Hardoi. DV-3 was isolated from the 1966 epidemic at Vellore and subsequently all 4 strains were isolated in 1968. DV -2 was the principal serotype circulating in northern India especially Delhi, Lucknow and Gwalior. Phylogenetic analysis of DV-2 strain in Delhi during 1996 were genotype IV .Earlier isolates during 1957, and 1967 belonged to genotype V. DV-2 genotype IV has replaced genotype V, which continues to circulate mutely in North India and has the prospective to re-emerge and cause key epidemics of DF and DHF.[4] A serological investigation done in Kanyakumari district of Tamil Nadu during 2003 dengue epidemic revealed DV-3 as the causative organism.[9]

DV-3 isolated were reported from epidemics in Vellore in the year 1966 and at Calcutta in the year 1983, Gwalior in 2003 and 2004, Tripur (TN) in 2010. A 2013 outbreak in Kolkata involved three serotypes DV1, DV3 and DV4.[5,8]

In Delhi uptil 2003, the predominant serotype was DV-2, but in the year 2003 for the first point in time all four dengue virus serotypes were found to co-

circulate in Delhi that altered it to a hyper endemic area and may be a reason for increased severity of DHF/ DSS.[4]

EPIDEMIOLOGY:

Agent

The dengue viruses are members of the species Flavivirus and family Flaviviridae. These diminutive (50 nm) viruses enclose a single stranded RNA as genome.[1,10] The virus is subclassified in four serotypes namely DEN-1, DEN-2, DEN-3, DEN-4.[1,10,11] All investigations undertaken to this day show that the four serotypes of dengue virus core are phylogenetically diverse, and frequently to the same extent as different "species" of flaviviruses.[11] The first dengue virus was taken from soldiers who became sick in Calcutta, New Guinea, and Hawaii.

The virus is spherical and has an isometric core of 30-35 nm diameter. The nucleocapsid (or) core (c) protein is intricate with the single stranded RNA. This is enclosed by a lipid bilayer. which forms the covering and contains matrix (or) membrane (M) protein. The total span of the enveloped virus is 45-60nm.[1,13,14]

The Dengue virus genome is 11,644 nucleotides in extent and has:

1. *Three structural protein genes* which encodes the nucleocapsid core protein (C), a membrane – associated protein (M) and an envelope protein (E).[1,13]
2. *Seven non- structural protein (NS) genes* – (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5).[1,13,16] Among the non structural protein, envelope glycoprotein NS1, is 45 kDa in size and is connected with viral haemogglutination and neutralization activity.[1]

Functions of structural proteins :

***C Protein* :** It is a 13 kDa protein. It contributes to group activation and is detected by compliment fixation test.

***E Protein* :** It is glycoprotein. It may help in anchoring of the virus to the host cell receptors. Antibody to E protein is protective.

***Pr M (M Protein*):** It has a molecular weight of 22 kDa. Its function is to stabilize polymerization of E protein.

Functions of the Non –structural proteins :

NS1 :

It is a membrane bound glycoprotein and is expressed on virus infected cells. It has replicative function. Antibodies to it are neither protective nor neutralizing.[20,22]

NS3 :

It acts as a virus specific protease along with NS2A.[20,22]

NS5 :

It is a virus polymerase.

As mentioned before, 4 serotypes of dengue exist. They have common antigens. They vary in their ability to cause disease.[20,22]

The Vector :

Aedes aegypti and Aedes albopictus are the two significant vectors of dengue[1].

Aedes (stegomyia) aegypti:

As the name suggests Aedes aegypti mosquito has it's origins in Africa, where it exists as a wild species procreating in forests free of humans. Subsequently the species adapted to the peridomestic environment. Slave trade and commerce during 17^{th} to 19^{th} centuries as well as World War II provided enough opportunities for the species to spread throughout the world. Other factors include rapid urbanization and population explosion, high scale of domestication and high attraction for human blood. Today Aedes aegypti is a cosmotropical species and it ranges between lattitudes 45^0N and 35^0S.[1,14]

Aedes (stegomyia) albopictus :

Aedes albopictus is a species originating in Asia, native to South East Asia and islands of the Western Pacific and the Indian Ocean. Nevertheless during the last couple of decades the genus has extended to continents like Africa, West Asia, Europe and the Americas. There is rising fear that Aedes albopictus can cause severe outbreaks of arboviral disease given that it is a proficient vector of atleast 22 arboviruses,especially dengue (all four serotypes) is more frequently transmitted by Aedes aegypti. [1,14]

The common breeding sites for Aedes aegypti are domestic containers, ornamental containers, discarded receptacles, flower pots and roof gutters.[13,14]

The mosquito breeds and thrives during rainy and post rain seasons. A higher atmospheric temperature results in proliferation of the Aedes mosquitoes, as well as high humidity.[1]

Their morphology includes black body with white stripes. They bite predominantly during morning and late afternoon hours. They predominantly lives indoors and are endophagic.They feed mostly on human blood (anthrophilic). The lifespan is around 1-4 weeks and eggs are not laid in clutches, which help in widespread dissemination and increased chance of survival and they withstand dessication for months. Transovarian transmission of the virus exists and this helps in maintenance during the interepidimic periods.[1,14]

Aedes species other than aegypti and albopticus transmitting dengue are Aedes polyneinsis, Aedes cooti and Aedes cutellans hebrideus.[13]

Host :

Dengue viruses have evolved from mosquitoes, tailored to non-human primates and afterward to humans in an evolutionary course.

The viremia amongst humans builds up to elevated titres, 2 days prior to the beginning of the fever and lasts 5-8 days following this through the febrile phase. During this phase the vector genus gets infected.[1,16]

The spread of the virus happens through the activities of the host (man) as the vector movement is very constrained.[1,16] The susceptibily of humans depends upon the immune status and genetic predisposition[1]. Together monkeys and humans are amplifying hosts and the virus is sustained by mosquitos transovarially by means of eggs.[1]

Transmission cycle:

It occurs in three different cycles.

1. **Enzootic cycle :**

A primitive sequence maintained by monkey- Aedes- monkey cycle is seen in South Asia and Africa. Virus is not pathogenic to the monkeys and viremia persists for 2-3 days. All 4 serotypes have been taken from monkeys.[1,16]

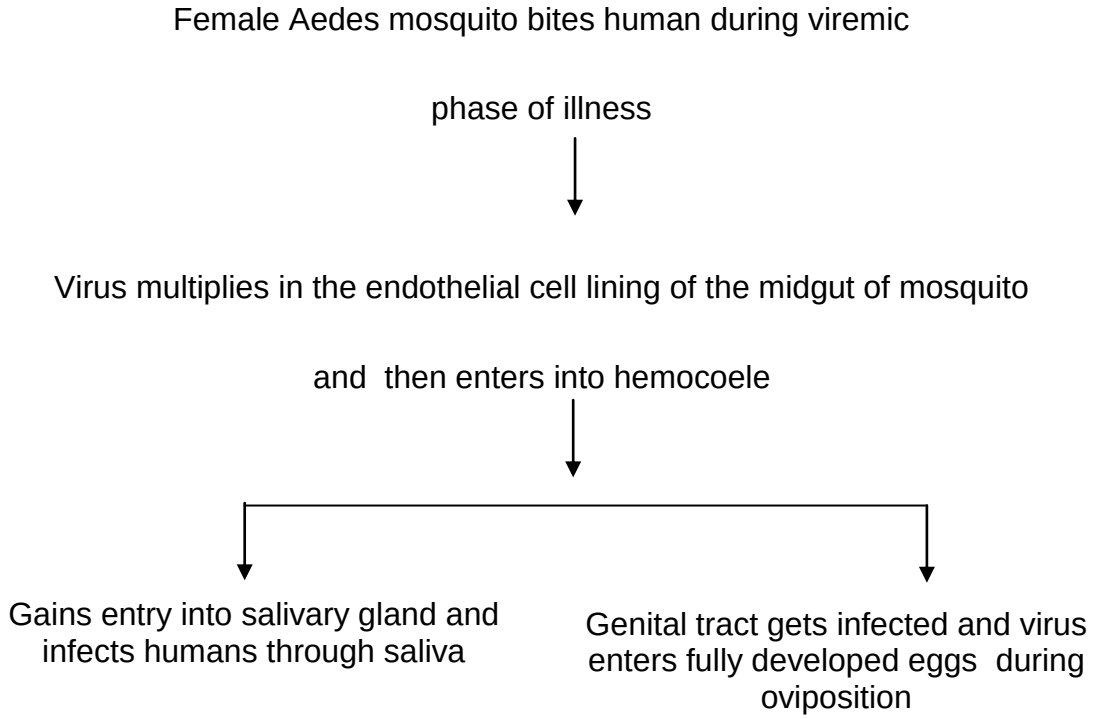
2. **Epizootic cycle :**

The dengue virus crosses over to non-human primates from neighboring human epidemics by vectors called bridge vectors. This was first observed in Sri Lanka during 1986-1987 among toque macaques which was proved by serological studies.[1]

3. **Epidemic cycle :**

This cycle is maintained through human- Aedes – human cycle in episodic/ recurring epidemics. All serotypes move and leads to hyperendemicity. In general Aedes aegypti has little inclination to oral infection by dengue virus but it's really strong affinity towards human hosts (anthrophily), multiple feeding tendencies and extremely domesticated habitats make it an competent vector.

Transmission of DF/ DHF: [1,17]



The existrinsic incubation period (ETP) extends from 8-12 days and mosquitos continue to be infected till the end of it's life. The Intrinsic Incubation Period (IIP) is from five to seven days.[1,17]

The Human Phase ;

Following bite of an infected mosquito, virus multiplies in the local lymphnodes and in 2-3 days spreads to the blood and various tissues. Virus circulates in the blood for 5 days. It also replicates in the skin, splenic, lymphoid cells and macrophages.[17]

Immunopathogenesis: [1, 16-29]

Dengue virus infection causes dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) , pathogenesis of which are not clearly unstated till date.[16] Several hypothesis has been proposed regarding the access of virus into host cells and its replication and virulence.[16,17]

Viral Entry and fusion with Target cells:

- Entry of flaviviruses is helped by numerous activities taking place at the host cell. The host cell has surface receptors and shows endocytic activity and triggers signals for infiltration.
- Flaviruses are internalized into host cell by coated vesicles and transported into endosomes. This process happens in an acidic Ph (6.5), optimally.
- Monocyte – macrophages are the primary target cells for dengue viruses.
- The virus E-proteins mediates the connection to cells, but the nature of host cell receptors remains obscure. Probable receptors include Fc receptors, glycosaminoglycans (GAGS) and lipopolysaccharide binding CD14 – associated molecules.

Dengue Virus Replication:[18]

- Virus enters the host cell by receptor mediated endocytosis as described earlier.
- Upon entry into host cell, endosomes are internalized and acidified, union of viral and vesicular membrane allows access of the nucleocapsid into the host cell cytoplasm and uncoating of the genome takes place.

- Translation of input strand occurs after which the virus switches from translation to synthesis of a negative – strand intermediate which becomes a template for the creation of multiple copies of positive – strand viral RNA (vRNA) ,subsequently produces high levels of viral proteins.
- This same process of translation produces multiple copies of structural protein capsid or core (C), premembrane (prM) and envelope (E) proteins.
- Progeny virions are thus assembled which are taken through the Golgi compartment and secreted.

Molecular basis of Immunopathogenesis: [16, 20, 22, 23, 24, 25, 26]

Several hypothesis for the pathogenesis of dengue virus infection have been projected. Among the several hypothesis antibody dependent enhancement (ADE) of infection has long been understood to play a key role.

This was proved with sera got before infection from children who later developed DHF/DSS and were more likely to reveal ADE in vitro than those who had only DF.

Effects of Dengue virus infection on Blood Cells:[16]

(1) Aberrant Immune Activation during dengue virus infection:

This theory was initially proved during a outbreak of dengue fever from November to December 1998 with serotype 3 in the nation of Southern Taiwan.

(A) Inversion of CD4/CD8 ratio:

- $CD4^+$ are in excess than $CD8^+$ T cells in peripheral blood of normal persons. In patients with DHF/DSS $CD8^+$ cells exceed that of $CD4^+$ cells. So that the ratio of CD4/CD8 cells declines to <1.
- The occurrence of CD4/CD8 inversion was seen more in patients with DHF/DSS than in patients with DF.

(B) Atypical lymphocytosis and Bandemia:

- The percentage of $CD4^{dim}$ and $CD8^{dim}$ monocytosis was higher or highest during day 6-7 in peripheral smear.
- The immature neutrophil elevation (Bandemia) happened during days 5-6 after the onset of fever.
- Atypical lymphocytosis reached the highest point on days 8-10 and then disappeared rapidly after day 12.
- Early activation of mononuclear cells was established by expression of the early activation marker CD69 on day 4 after fever commencement. The appearance of atypical lymphocytes and active changes in the CD4/CD8 ratio suggest that aberrant immune activation does take place during dengue virus infection.

2) Cytokine over production during Dengue virus Infection:

- As mononuclear cells overactivation happens through acute dengue infection, it is anticipated that elevated levels of cytokines can be seen in the serum
- Pro inflammatory markers include:

- T-cell activation markers – soluble CD4, soluble CD8, IL-2, soluble IL - 2 receptors and IFN-γ
- Monokines - IFN- γ, TNFα and GM-CSF.

These markers were seen to be higher in DHF/DSS patients than in DF patients.

- Elevated levels of inhibitory cytokines – IL-10, soluble receptors of sTNFR I, and sTNFR II
- IL – 6 has twin roles as both a proinflammatory and an anti-inflammatory mediator. Its kinete analysis showed disparity at different time points and in different persons.
- These fluctuations in IL-6 imply that when a host responds to dengue virus infection through production of proinflammatory cytokines, at the same time there is also production of inhibitory cytokines to counter the inflammation.
- Cytokines may cause cell activation synergistically or antagonistically, the overall effect will depend on the balance between various cytokine actions.

Thrombocytopenia and Anti-platelet Antibodies:[16,52,53]

Thrombocytopenia is frequent in DF and almost always found in DHF/DSS. The probable reasons of thrombocytopenia in dengue fever are:

(1) Dengue virus – induced bone marrow suppression leading to low platelet synthesis and thrombocytopenia

(2) Platelet destruction induced by DV antigen attached to human platelets without immune mediated reaction.

(3) Anti-platelet autoantibodies (predominantly IgM class) induced platelet lysis and it also inhibits ADP-induced platelet aggregation. The affinity towards these auto antibodies is enhanced in secondary dengue infection.

So far this hypothesis holds good and has enough animal models and human studies.[52,53,66]

(4) A modulation of endothelial cells by the infection of DV to the cell, was suggested as one of the cause of thrombocytopenia.[52]

Dengue Virus – Induced Vasculopathy:[16,19]

- The most typical feature of DHF/DSS and the best pointer of disease severity is plasma leakage
- Plasma leakage is caused by a faulty increase in capillary permeability and manifests as combination of hemoconcentration, pleural effusion, or ascites.
- Plasma leakage occurs systemically, moving ahead quickly and usually resolves in 1-2days in patients who receive sufficient fluid resuscitation.
- Although perivascular oedema is apparent no destruction of vascular endothelial cells are usually obvious.
- Functional modification of endothelial cells is most likely caused by effects of cytokine or mediator release such as IL-6, IL-8 and RANTES.
- In vivo studies have demonstrated destruction and apoptosis of endothelial cells especially by certain strains of DENV-2 (Strain PL 0046)
- Dengue virus infected endothelial cells are able of activating complement and expression of adhesion molecules such as ICAM-1. The expression of ICAM-1 along with the production of chemokines IL - 8 and RANTES

increases the adherence of PMN and mononuclear cells to vascular endothelium and increase in vascular permeability and thrombomodulin release.

- Thrombomodulin is a marker of endothelial damage and its levels were found to high in patients with DHF/DSS indicative of endothelial structural damage.
- It is concluded that direct viral cytopathic effects, immune mediated endothelial damage by leukocyte recruitment and anti-dengue antibodies can cause structural injury to infected endothelial cells.

As endothelium plays a central role in maintaining hemostasis,endothelial damage may result in procoagulant / anticoagulant imbalance, sequestration of platelets by the activated endothelial cells contributing to thrombocytopenia will increase bleeding tendencies apart from plasma leakage

Dengue Virus – Induced coagulopathy:[16,22,23,24,26,27,28]

The pathogenesis of bleeding in DHF is unclear even though well recognized coagulation disturbances exist.

- Clinical manifestation ranges from a positive torniquet test, ecchymoses, skin petechiae to epistaxis and gum bleeding to severe gastrointestinal hemorrhage.[25]
- Hemorrhage results from two mechanisms
  - (i) Severe thrombocytopenia and associated platelet dysfunction and endothelial dysfunction
  - (ii) Coagulopathy including DIC.

(iii) Hemostasis is maintained by the equilibrium between coagulation and fibrinolysis.

- **Coagulation system** - consists of intrinsic as well as extrinsic pathways

↓ Activation

Thrombin formation

↓ Converts

Fibrinogen – Fibrin

↓ Leads to

Formation of thrombus

- **Fibrinolytic system**

(facilitates fibrinolysis – clot lysis)

Plasminogen (proenzyeme)

↓

Activated by various types of

Plasminogen activators

(Principal endogenous activator is tissue – type plasminogen activator (tPA))

↓ Conversion of plasminogen to

Active plasmin enzyme

↓ Breaks down

Fibrin to fibrin degradation products (FDP)

- tPA is inhibited by plasminogen activator, inhibits (PAI-1) which is synthesised by liver, platelets and endothelium.
- Coagulation activation

↓ Triggers

Secondary activation of fibrinolysis

↓ Rapidly shut down by

Discharge of large amount of PAI – 1

- In acute dengue infection, thrombocytopenia and altered liver functions leads to reduced PAI-1 secretion and in turn leads to bleeding disorder.
- In addition there is defect in intrinsic pathway of coagulation leading to a prolonged aPTT.
- Summarising the coagulation disorder in DHF/DSS, factors which leads to a greater risk of hemorrhagic tendencies are

(i) Thrombocytopenia

(ii) Endothelial Dysfunction

(iii) Raised tPA activity

(iv) Subnormal PAI-1 activity

(v) Prolonged aPTT.

Pathogenesis of liver cell Dysfunction:[16,29,61]

The clinical confirmation of liver involvement in dengue infections include the presence of hepatomegaly and augmented levels of liver enzymes.[61]

- Several studies have found the elevation of aminotransferase to the tune of >90% (AST) and >80% (ALT) in cases of DF, DHF/DSS.
- The elevation of transaminases tend to be higher in patients with DHF/DSS than in DF.[16,61]
- The levels usually return to normal range by 14-21 days after infection.[61]
- Fulminant heptitis and hepatic failure have been recorded in patients with DHF/DSS and carries a poor prognosis.[29]
- There is no evidence chronic liver cell failure in patients with DF/DHF.[61]
- The level of AST appears to get elevated more than ALT, which is vice versa in other viral hepatits.[61]
- The elevated AST levels usually returns to normal more quickly than ALT levels this may be due to shorter half-life of AST than ALT.[61,29]
- Histological changes in hepatitis associated with DF includes steatosis,microvesicular, hepatocellular necrosis, Kupffer cell hyperplasia and destruction, Councilman bodies and cellular infiltrates at the portal tract.
- Hepatocellular necrosis in DF usually affect the midzonal and sometimes the centerilobular areas. The cause for this phenomenon is that cells in midzonal area are more susceptible to anoxia or to products of immune response (Cytokines and chemokines) or dengue virus preferentially infects cells in this zone.[61,16]

- Inflammatory cell that typically infiltrates the liver during DF are mononuclear cells.
- The Dengue virus has the capability to infect both hepatocytes as well as Kupffer cells but replication occurs efficiently in hepatocytes only.
- The destruction of hepatocytes in either due to direct involvement of dengue viruses leading to apoptosis or may be immune mediated destruction.[16,61]

Clinical Manifestation:

Dengue virus illness may be asymptomatic or may lead to undifferentiated febrile sickness, dengue fever (DF) or dengue hemorrhagic Fever (DHF) plus dengue shock syndrome (DSS). [1,7]

Infection with one dengue virus serotype gives life long immunity to that similar serotype, but there is only a short period of cross protection for the other different serotypes. The clinical symptoms depends on the virus strain and host factors like age, immune status etc.[1]

Undifferentiated fever :

Primary dengue infection possibly will present with a fever indistinguishable from other viral syndromes with accompanying maculopapular rash and respiratory or gastro intestinal symptoms. [1]

Dengue fever (DF) :

It is seen most commonly in older children, teenagers and grownups. It presents as illness, with sometimes with biphasic fever accompanied with headache, myalgias, arthralgias, rashes, thrombocytopenia and leucopenia The

severe manifestation of the above mentioned symptoms have given rise to the terminology "break – bone fever". [1,3,7]

The intrinsic incubation perioid is usually 4-6 days with a range of 3-14 days. The temperature is usually between 39^0C-40^0C and lasts for 5-7 days. This is accompanied by myalgias, arthalgias, retro orbital pain, photophobia. Other symptoms includes nausea, anorexia, constipation, diarrhoea, abdominal pain,sore throat and depression. The symptoms may continue from quite a few days to weeks.[1,3,7]

The rash manifests as diffuse flushing or fleeting eruptions in the first 2-3 days over face, neck and chest. A evident rash may be maculopapular or rubelliform, appears between 3-4 days and by the end of febrile phase or right away after defervesence .This widespread rash fades and localized clusters of petechiae become visible over the dorsum of the feet, over the legs , on the hands and arms.[1,7]

The relative period and relentlessness of the illness varies between individuals and between one epidemic to another. Convalescence may be of short duration but sometimes prolonged and extends for several weeks and is followed by marked asthenia and depression. Bradycardia is frequent during this period. Hemorrhagic manifestations are unusual in dengue fever(DF).[1,3,7]

Dengue Hemorrhagic fever and Dengue shock syndrome :

Dengue hemorrhagic fever (DHF) is for the most part frequent in children less than 15 years old in hyperendemic areas associated with repetitive dengue infection.[1]Abnormal hemostasis and plasma leakage are the hallmarks of DHF. Thrombocytopenia and increasing hematocrit/ hemoconcentration are invariable

findings prior to the subsidence of fever/ onset of shock. It is frequently associated with secondary dengue infection, but primary infection with DEN-1, and DEN-3 as well as infants can manifest this from of dengue syndrome, without prior infection. [1,3,7,19]

The initial clinical manifestation of DHF/ DSS resembles symptoms of DF. Illness begins with high fever which lasts for 2-8 days before diminishing to normal or sub normal levels. Constitutional symptoms include anorexia, headache, muscle or joint pains and throat pain, with injected pharynx.[1,7,19]

Epigastric uneasiness, tenderness at the right costal margin and generalised abdominal aches are frequent. A positive torniquet test (≥10 spots/ square inch), the most frequent hemorrhagic occurrence, could be seen in the early febrile phase.[1,7,19]

Rash as described in dengue fever may develop during febrile phase and during convalescence.

Bleeding in the form of epistaxis and bleeding gums are less frequent. Mild gastro intestinal hemorrhage is infrequently seen.

Liver is typically palpable during premature febrile stage, varying from just palpable to 2-4 cms beneath the right costal margin. Hepatomegaly is seen more frequently in shock cases. Splenomegaly is observed more frequently in infants under twelve months.[1,19]

The grave phase of DHF i.e. the phase of plasma leakage, begins just about the shift from febrile to the afebrile phase .Confirmation of plasma leakage includes pleural effusion and ascites. Gall bladder oedema has been seen to herald plasma leakage.[1,3,7,19]

In mild cases of DHF, all signs and symptoms subside after the fever abates. Patient more often than not recover either unexpectedly or after fluid and electrolyte treatment.[1,7,19]

Dengue shock syndrome :

In moderate to severe cases of D.H.F the patients state deteriorates a few days after the commencement of fever. There are warning signs such as persistent vomiting, abdominal aches, reduced oral ingestion,lassitude and irritability.[1,2,7,25]

Hypotension and Oliguria :

By the time or shortly after the febrile phase ends there are evidence of circulatory collapse.[1,7,25] The skin becomes cold, blemished and congested, circum- oral cyanosis is often seen. The pulse becomes feeble and fast.[1,7,25]

The shock is manifested by a weak and rapid pulse with the reduction of pulse pressure (≤ 20mm Hg), an augmented diastolic blood pressure (or) hypotension. Signs of decreased tissue perfusion capillary refill1) <3 seconds 2)Cool, damp skin and3) agitation. If appropriate intervention is not done patient may deteriorate into a profound shock with pulse and blood pressure becoming unnoticeable (grade 4 DHF) .Shock is reversible if adequate treatments with volume replacement and fluid resuscitation is given, otherwise the patient may die within 12-24 hours. [1,7,25]

Criteria for clinical Diagnosis of DHF/ DSS:[1,2]

1. Fever of high grade / lasting for 2-7 days.
2. Any of the subsequent hemorrhagic manifestations – positive tourniquet test (the commonest), petechiae,purpura, ecchymosis, epistaxis, bleeding gums and hematemesis and /or melena.
3. Shock,characterised by tachycardia, low tissue perfusion with feeble pulse and lessened pulse pressure (20mm Hg or less) or hypotension with presence of cool, sweaty skin and / or agitation.
4. Enlargement of liver (is seen) at some point of the disease in 90%-98% of kids

Clinical Laboratory Findings in Dengue fever (DF):[1]

- Total WBC count is more often than not normal at the commencement of fever followed by leucopenia.
- Platelet count is frequently normal. Mild thrombocytopenia in the range of 1,00,000 to 1,50,000 cells/mm^3 is common. Around 50% of patients with DF will have platelet count of <1,00,000 cells/mm^3 .Severe thrombocytopenia (<50,000 cells/mm^3) is usually rare.
- Mild elevation of hematocrit (~10%) may be found as a consequence of dehydration.
- Biochemistry tests especially liver enzymes (AST,ALT) may be slightly elevated.

Clinical Laboratory findings of DHF:[1]

- **WBC count :**

  It can be normal with predominant neutrophils in the early febrile phase. Then it may drop reaching a lowest point towards the end of febrile phase. A total WBC count of $\leq$ 5,000cells / mm^3 and a ratio of neutrophils to lymphocyte (neutrophils < lymphocytes) is helpful to foresee the critical period of plasma leakage. This result precedes thrombocytopenia or rising hematocrit. A relative lymphocytosis with increased atypical lymphocytosis is frequently observed by the end of febrile phase.

- **Platelet count:**

  Platelet counts are normal during the beginning of the febrile stage. A sudden fall in platelet count below 1 lakh occurs by the ending of the febrile phase. The level of platelet count correlates with the severity of DHF.

- **Hematocrit:**

  It may be normal during early febrile stage. A trivial increase may be because of high fever and dehydration. Hemoconcentration or rising hematocrit by 20% from the baseline is a objective evidence of plasma leakage.

- Activated partial thromboplastin time (aPTT) and prothrombin time (PT) are lengthened in about one third and half of DHF cases respectively. Thrombin time is delayed in severe cases.

- **Other findings:**
  - Hypoproteinemia / hypoalbuminemia as outcome of plasma leakage
  - Hyponatremia

- Elevated ALT – AST usually at levels $\leq$200 IU/L with a ratio of AST:ALT >2.
- Hypocalcemia is usually seen in most cases of DHF, the level is lower in Grade 3 and 4.
- Metabolic acidosis is often found in patients with prolonged shock.
- Blood urea and creatinine is also increased in patients with prolonged shock.

Thrombocytopenia(100,000 cells/mm^3 or less) and hemoconcentration or a increasing hematocrit $\geq$ 20% from the usual values are adequate to establish a clinical conclusion of DHF. In cases with shock, a elevated hematocrit and marked thrombocytopenia hold up the diagnosis of DSS.[1,2]

Laboratory Diagnosis :

Swift and precise diagnosis is of vital importance for [1,31]

i) Epidemiolgical observation
ii) Clinical managing
iii) Vaccine trials
iv) Research

Early diagnosis is of paramount importance in successful management of dengue fever and identifying circulating serotypes/ genotypes during interepidemic phase for use in forecasting possible outbreaks.[32]

Blood collection in Tubes or Vials :[1,32,35]

- Withdraw 2-10 ml of venous blood with aseptic precaution.
- Labelling should include patient's name, identification number and data of collection.
- Use vaccum tubes or disinfected vials with screw caps and gasket.
- In anticipated delay of > 24 hours separate the serum and store frozen.

Specimens : Collection, storage and shipment:[1,32]

- Blood is collected as paired specimens.
- Collection specimens are divided according to the different time intervals they are acquired.

i) Acute phase specimen (S1) :

They are collected as soon as possible after the onset of illness (0-5 days of onset), stored at -70^{0}C, and transported in dry ice

ii) Convalescent phase specimen (S2)

They are obtained during release from hospital (or) in the occurrence of a fatal outcome.

iii) Late Convalescent phase specimen(S3) :

They are collected 7-22 days after the acute phase serum was drawn. Both S2 and S3 are stored at minus 20^{0}C and shipment done in Frozen or ambient state.

iv)Tissue sample:

They are collected as soon as possible after death , stored at minus 70^{0}C or in formalin, transported in dry ice.

Shiping done with wet ice for blood and dry ice in case of serum. Shiping should stick on to national international procedures for transportation of infectious substances

Diagnostic Tests :[1]

- Virus Isolation – Serotypic/ genotypic classification
- Virus Nucleic acid finding
- Virus antigen recognition
- Immunological response based tests – I_gM and I_gG antibody assays
- Study for hematological criteria

Diagnostic test and phases of disease: [1,31,32]

- **Viremia**

  Typically occurs 2-3 days prior to onset of fever to 7 days of illness.

  During this phase dengue virus, nucleic acid and viral antigens (NS1) can be detected.

- **Antibody response**

  i) I_gM antibody – 3-5 days of onset of illness, rises upto 2 weeks to imperceptible levels after 2 or 3 months

  ii) I_gG antibody – demonstrable by end of week and remains elevated for many years

Diagnostic methods for Detection of Dengue Infection [1,35]

Dengue early stages of disease, virus segregation, viral nucleic acid or antigen discovery can be used and at the end of acute stage immunological tests are the preferred method for analysis.

A) Segregation of virus :[35]

- Ideal sample collected within six days of illness
- Preferred specimens – Acute phase serum, plasma, washed buffy coat, autopsy tissues like (liver specimen, lymph nodes ,thymus) ,live mosquitoes
- Mosquito cells culture followed by live mosquito innoculation are the most preferred methods .Innoculation of suckling mice if the former methods are not available.
- Mosquito cell lines C6/36 or AP61 are the host cells of preference for segregation by dengue viruses.
- Preferred : Mosquitoes include in order of sensitivity : Toxorhynchites amboinensis, Ae.aegypti and Ae.albopictus

B. Viral Nucleic Acid Detection:[35]

The primary investigation used is RT- PCR. The sensitivity for the method is 80-100%. RNA is heat labile and therefore specimens should be handled carefully. Methods comprise:

- Reverse transcriptase – polymerase chain reaction (RT-PCR)
- Nested RT- PCR
- One Step multiplex RT- PCR
- Real time RT- PCR
- Isothermal amplification method
- Loop mediated amplification (LAMP) PCR

These tests need a BSL 2 lab with gear for molecular biology and trained professionals. The newer modalities can be used to identify different serotypes, early detection of infection and include high sensitivity and specificity.

c) Viral Antigen Detection [1,37,38,39]

Out of the dengue non- structural proteins, the NS1 antigen detection has revolutionalised early diagnosis of dengue fever[1].

- Commercial availability of NS1 detection kits are there scince 2002.[38]
- NS1 antigen is a highly preserved glycoprotein, produced in both membrane bound and secretary forms, is abundant in the serum of patients during early stage of DEN-V infections. [1,38]
- NS1 antigen shows up as early as day 1 after the onset of fever and persists upto 9 days.[1,38,44]
- In secondary dengue infections antibodies clear NS1 from circulation within 5-7days. [37,39,45]
- Detection of NS1 antigen by ELISA method is preferable.[1,45]
- Immuno enzymes and immunochromatographic techniques (i.e. rapid tests) are also available at present. [1,38]

Immunological Response and Serological Tests :[1,37]

Five fundamental tests are used for the diagnosis of dengue infections they are :

i) Hemagglutination – inhibition (HI).

ii) Complement fixation (CF)

iii) Neutralization test (NT)

iv) I_gM capture enzyme linked immunosorbent assay (MAC- ELISA Indirect I_gG ELISA)

For diagnostics other than those that detect IgM,unequivocal corroboration depends on a noteworthy (four fold or greater) rise in specific antibodies amid acute phase and convalescent- phase serum samples.

1. I_gM antibody capture Enzyme – linked Immunosorbent assay (MAC ELISA)[1,31,35,41,42]

MAC ELISA is a simple and rapid test which has become extensively used in the last few years.[1]

It is based on the detection of the dengue specific I_gM antibodies in the test serum by capturing them out of solution using anti-human I_gM that was beforehand bound to the solid phase.

- Anti Dengue I_gM antibody is usually detectable by day 5 of the illness, a little earlier than I_gG.
- IgM antibody titres are significantly higher in primary than secondary
- Levels peak at around 2 weeks after the start of symptoms and in most patients it decreases to an untraceable levels by 60 days. It might persist upto 90 days.
- A positive I_gM MAC – ELISA is suggestion of recent dengue infection.
- It is particularly helpful for hospitalized patients who are usually admitted at a last phase of illness.

I_gG ELISA :[1,31,35]

It is an easy to perform test and primarily used it differentiate between primary and secondary dengue infection.

- I_gG ELISA is extremely non- specific in terms of cross reactivity among other flavi viruses.
- I_gG antibodies starts rising at the end of first week and remains elevated for many years.

I_gM/ I_gGRatios :[1,31,35]

- It is used to differentiate between primary and secondary infection.
- Primary infection is defined by a capture I_gM/ I_gG ratio > 1.2
- Secondary infection if the ratio is < 1.2

Hemagglutination Inhibition Test (HI) :[1,31,35]

- It is sensitive, easy to perform and was used previously for routine serological diagnosis of dengue infection.
- HI antibodies persist for > 50 years and are best for sero- epidemiologic studies.
- Main drawback is that the test lacks specificity.

Complement fixation Test (CFT) :[1,31,35]

- CFT is used to diagnose current infection.
- Its use is restricted because it is complex to perform and requires highly skilled personnel.

Neutralisation Test (NT) :[1,31,35]

- It is the most sensitive and specific serological test.
- The disadvantages are that this test is expensive, time consuming and needs expertise.
- It finds immense use in the making of vaccines and in their efficacy trials.

MANAGEMENT

There is no specific therapeutic agent for dengue virus infection. High dose of steroids have no role in recovery. There is no benefit of using I.V. immunoglobulin. There is no antiviral drug specific to its treatment.

Management of dengue fever:[1,7]

Symptomatic treatment with bed rest, antipyretics and analgesics.Aspirin is avoided as it may aggravate gastritis or bleeding tendencies. In children Reye's syndrome may be a grave complication. Antibiotics do not play a role here.

Oral rehydration is essential. ORS (oral rehydration solution) is suggested for patients with excess sweating, vomiting or diarrhoea to avert dehydration.

Food is given according to appetite following recovery from the fever. The patient is monitored for 2 days for shock which is a complication during this period.

Management of dengue haemorrhagic fever:[1,7]

Prognosis depends upon early recognition of plasma leakage which can be assessed by rise in hematocrit level. Repeated monitoring may be required. Patient should be admitted when rise in hematocrit is 20% or more, single hematocrit is more than 40% ,platelet count of 50,000/mm^3 or less, presence of unprompted haemorrhage or signs and symptoms of shock or oliguria or circumoral cyanosis. Treatment should include antipyretics like paracetamol. Oral fluids are to be given to avoid dehydration. IV fluids should be given along with constant monitoring of haematocrit level and regular estimation of vital

signs like urine output to circumvent over infusion and hence circulatory overload. To prevent fluid overload, fluid therapy is stopped when hematocrit drops to approximately 40% and clinical signs and urine output improves.

Management of dengue shock syndrome:[1,7]

Here volume replacement by intravenous fluid administration helps to expand the plasma volume. Close observation with constant monitoring of vital signs, urine output and hematocrit is important.

FFP or concentrated platelet transfusion can be initiated when disseminated intravascular coagulation leads to substantial bleeding. Theoretically the thrombocytopenia is a risk issue for haemorrhage. The threshold for prophylactic platelet transfusion is 10,000/mm^3 in non dengue cases. It has been recommended that since there is no definite therapy for DHF/DSS, patients bleeding tendency and a platelet count of less than 20,000 – 25,000 / mm^3 may be empirically transfused platelets. If clinically indicated fluid electrolyte replacement should be done.

Platelet Transfusion in Dengue fever:[1,2,46,47,48,49]

Thrombocytopenia is one of the important finding in dengue fever. It is almost always associated with dengue hemorrhagic fever. The medical fraternity internationally recognizes the role of platelet transfusion in the management of hospitalised dengue patients, but the precise indications and situations in which transfusion is done may vary. There is always a dilemma regarding platelet transfusion in a non - haemorrhagic patients with dengue fever. A platelet count of <1,00,000 cells/cu.mm with evidence of bleeding is almost always an indication for platelet transfusion in a patient with dengue

fever. There are no clear cut guidelines regarding platelet transfusion in our scenarios.

In general a platelet count of <50,000cells / cu.mm is associated with minor risk of any form of bleed, and the risk increases if the platelet count drops by 10,000-20,000 cells /cu.mm and the risk of major bleeding including hollow viscous bleed is substantially more. The risk of bleeding is more when thrombocytopenia is associated with coexsisting coagulopathies, liver disease, platelet inhibiting drugs and infection. The minimum threshold for platelet transfusion is <10,000 cells / cu.mm in otherwise stable patients as a general rule.

As it is understood not only the quantity of platelet is altered in DF, also the quantity i.e. the functional part is also affected they may lead to an increased risk for haemorrhage in patients with dengue fever. Many studies have shown including the WHO guidelines for prevention and control of DF that the level of thrombocytopenia and risk of hemorrhage in DF has poor correlation, but some studies have enough evidence that the risk of hemorrhage in substantially raised with the level of thrombocytopenia, especially if the platelet count drops below 20,000 cells / cumm.

Factors affecting the prognosis of Dengue fever :[1]

1. Existence of enhancing and non-neutralizing antibodies increases the severity.
2. Age:Suceptibility of DHF / DSS falls considerably after age of 12 yrs.
3. Sex: Females are very often affected than males
4. Face: Caucasians are more often affected than blacks

5. Sequence of infection: Serotype 1 followed by Serotype 2 infection seems to be more unsafe than Serotype 4 followed by Serotype 2
6. Infecting serotype : Type 2 is in fact more perilous than the other serotypes

Dengue vaccines:[1-3,7]

The vaccine for dengue is in the process of development and trials. No ideal vaccine has been produced. Any vaccine synthesized must be tetravalent as preexisting heterotypic dengue virus antibody becomes a risk factor for DHF.

The different types of vaccines are

1. Live attenuated dengue vaccine
2. Chimeric dengue vaccine

These are synthesized using recombinant DNA technology. Here a chimera is created in which the structured protein genes for target antigens of a virus was replaced by equivalent genes of another virus. Hence a chimera was created with required attenuation phenotypes and expression of target antigen.

a. Chimeric yellow fever/dengue virus (ChimeriVax-Den) was developed. The vaccine expressed the premembrane (PrM) and E genes for dengue virus 2 in an yellow fever virus (YFV – 17D) genetic background.
b. ChimeriVax – DEN, was constructed and represented DV serotype 1 to 4 by electroporation of vero cells with RNA transcripts made from viral cDNA.

DNA Dengue vaccines:[1,2,3]

Here PrM, the genes of dengue virus – 2 were cloned into different eukaryotic plasmid expression vectors. The vaccine obtained was tested by introducing into BALB/c mice intradermally.Rhesus macaques were also used.

Case studies in Dengue fever :

Bandyopadhyay B,Bhattacharya I,Adhikary S,Konar J,Dawar N et.al.[55] in their study titled "A comprehensive study on the 2012 Dengue fever outbreak in Kolkata, India" done at the Calcutta School of Tropical Medicine selected 62 cases of clinically suggestive dengue fever to evaluate the performance of the newly introduced dengue NS1 antigen detection test (Pan Bio – Australia NS1 ELISA Kit) who reported early just after the onset of fever and were followed up to 14 days of illness. Out of the 62, 38 of the patients tested positive for NS1 antigen and 24 cases were found negative for NS1 antigen. Out of the NS1 positive patients, 24 were also found to be positive for dengue IgM antibodies and rest 14 of the were IgM negative. The possible causes include false positive NS1 antigen test or may be secondary dengue infection where the IgM antibody titre tend to be undetectable. Out of the 24 negative cases 6 of them subsequently become positive for IgM ELISA during follow-up. This shows that NS1 antigen positivity should be correlated clinically for reasonable accurate diagnosis of DF.

Hati AK[56] in his work quoted “Studies on Dengue and Dengue Hemorrhagic fever (DHF) in West Bengal state, India” at a tertiary care hospital in Kolkata, screened a total of 874 patients with acute febrile illness during August to November 2005 dengue epidemic were quantitatively analysed for dengue IgG and IgM antibodies using the FVD Microwell ELISA dengue fever test kit found that 52.6% had only IgG antibodies positivity, followed by 8.9% only had IgM antibodies positive, followed by 16.8% had both IgG and IgM positivity. 21.6% tested negative for both IgG and IgM ELISA. Out of these 10%

developed DHF and the case fatality rate was 8.5% among patients with DHF the out break was caused by serotype DEN – 3.

Tewari KN,Tuli NR,Devgun SC[57] in their retrospective study titled"Clinical profile of dengue fever and use of platelets in four tertiary fever hospitals of Delhi in the year 2009" obtained data by observing the formats and case records from 3 private multispecialty nursing areas within Delhi. A Total of 230 seropositive cases out of 426 patients of Dengue fever / Dengue hemorrhagic fever admitted between July and December of 2009 were randomly selected from MRDs of these hospitals. The inclusion criteria were seropositivity for DF/DHF by NCDC. Exclusion criteria were sero negativity and those cases where consent was denied by the hospital authorities. The age of these patients who were included in this study group were between 6 years and 82 years. The mean age for males was 30.4 years and 39.0 years for females. Out of the 230 cases studied, 163 (70.8%) had dengue fever (DF) and 67 cases had DHF. Fever was almost always present in all cases who got admitted. Other symptoms frequently encountered were vomiting (36%), pain abdomen (34%), headache (27%), myalgia (24%), nausea (19%), common bleeding manifestations were epistaxis, haematemesis and melena. In patients with DHF, hepatosplenomegaly with pleural effusion and ascites were the commonest finding (70.5%). Out of the DHF group, 3 patients (4.4%) manifested DHF class III (DSS). Platelet transfusion was done in 80 cases (48.7%) of DF group and 50 cases (73.5%) of DHF. 141 patients (82.6%) of DF patients had platelet count of 1,00,000. Minimum platelet count observed was 2,000 followed by 3,000 in cases of DF who didn't have any serious bleeding manifestations. Minimum units of platelets transfused is one and

maximum was 16 units with an average of 4.23 units. Platelet transfusions were not done as per international guidelines but was rather administered as per the severity of thrombocytopenia and in the anticipation of impending bleed. The positive outcome regarding platelet transfusion is that the average hospital stay for patients who received were 4.9 days compared to those without transfusion (5.3 days) with a significant p value (p = 0.0416). A total of 2 deaths were encountered in DHF group.

Raju BJ,Rajaram G[58] in their study titled "Prevalence of Dengue fever and Dengue Hemorrhagic Fever in Government General Hospital,Tirupathi" which was done at Department of Microbiology, S.V.Medical College, Tirupathi over a period between August 2007 to July 2008, screened a total of 200 cases who got admitted with clinical features suggestive of DF by testing their serum for dengue IgM antibodies using IgM capture ELISA test. Out of 200 patients 121 were male and 79 were females. Out of the 200 cases, 75 (37.5%) tested positive for dengue IgM ELISA. The commonest presenting symptoms are as follows fever (100%), headache (92%), myalgias/arthalgias (85.5%), nausea and vomiting (84.5), Pedal oedema (75.5%), skin rash (69.5%), pain abdomen (55.0%) conjunctival congestion (41.0%), Hepatosplenomegaly (30.0%), altered sensorium (28.5%), Retrobulbar pain (26.5%), Ascites (21.5%), Hemorrhagic manifestations (11.0%)

Khan AK, Sitwat A, Masood N, Solangi NM, Shaikh TZ et al[69] in their retrospective study of patients with acute febrile illness, concluded that 50% of patients presented with typical features of dengue fever were aged between 13-70 years. Only 40% were dengue proven. Out of these 18 had DF and 2 of them had DHF. Typical clinical features like fever with chills in 16 (80%),

Myalgia (10%), headache in 10 (50%), vomiting in 12 (60%), , pharyngitis in 7 (35%), rash in 5 (25%) and bleeding manifestations in 5% patients.

Lt.Col.Banerjee M, Lt.Col.Chatterjee J, Lt Col Choudhary GS, Col Srinivas V, Brig.Katari V.K [63] in their observation of 50 clinically inspected dengue cases concluded that 27 cases (54%) were confirmed positive by IgM antibody assay (ELISA). Commonest clinical features were fever with rash (85%) retroorbital headache (63%) myalgia (81%), hepatomegaly (15%) and hepatosplenomegaly (7%), Hemoglobin < 9.5gm% (11%), thrombocytopenia of <1,000,00 cells/cumm in 19%, leucopenia was not observed in this study.

Jain A, Shah A, Patel P, Desai M, Somani S, Parikh P et.al[65] in their study of 56 patients with dengue fever mostly among medical professionals in a tertiary care hospital in Ahmedabad has found fever followed by myalgia as commonest symptoms. the most common predictor of severity were raised hematocrit of >40% and a low platelet count of <50,000 cells/cu.mm. The mortality rate was 1.7%

Lin SF, Liu HW, Chang CS et al[67] in their study of patients with dengue fever in a Chinese hospital observed that leucopenia was present in 76% of cases and a thrombocytopenia of <1,00,000cells /cu.mm in 54% of the cases. Leucocyte count normalised at the 5^{th} – 6^{th} day after the onset of fever and thrombocytes reached nadir by 5^{th} – 7^{th} day after fever onset. Bone marrow studies in these patients revealed mild hypocellularity in the acute stages and normocellularity during the convalescent stage i.e. after I week post febrile phase.

Turbadkar D, Ramachandran A, Mathur M et al[68] in their retrospective study named "Laboratory and clinical profile of dengue: A study form Mumbai". did a screening of 3,677 clinically suspected cases of dengue fever at a tertiary care hospital by rapid test for antibodies against dengue. Out of these 503 (13.67%) sample tested positive which included positivity for IgM antibodies alone 288 (57.25%) (or) for both IgM and IgG antibodies 107 (21.27%). Those cases with IgG alone being positive were not taken into consideration as it suggested a remote infection .Only 212 out of 503 cases tested positive by IgM ELISA method the period of study was between January 2004 to November 2007. The peak incidence of cases on monthly basis was between July and November that suggests later part of monsoon as well as the post-monsoon period. Fever was the major clinical symptom (100%), followed by myalgia (25.0%) and headache (13.9%), thrombocytopenia (platelet counts <75,000 cells/cu.mm) were noted in 386 cases (76.74%), 68 cases (17.6%) has counts below 20,000 cells/cu.mm, 241 (62.4%) had counts between >20,000 – 50,000cells / cu.mm and 77 (19.9%) had counts between 50,000 – 75,000 cells/cu.mm.

Mandal SK, Ganguly J, Sil K, Chatterjee S, Chatterjee K, Sarkar P et al [70] in their study titled "Clinical Profiles of Dengue fever in a teaching Hospital of Eastern India". This study was conducted in a territory care hospital. A total of 74 MAC ELISA positive dengue cases were studied. The most common clinical manifestation was fever (100%) followed by headache (62.16%). Atypical manifestation like transaminitis and neurological manifestation were present in 83.83% and 11.0% cases respectively.

Fatima S, Abeddin MA, Firdous F [71] in their study titled "To assess the severity of dengue fever in patients attending a tertiary care teaching hospital using WHO grading system" at Deccan college of Medical Sciences screened a total of 494 patients with suspected dengue fever were screened with ELISA IgM, IgG and MICROLISA test for NSI antigen diagnosed 131 cases (26%) as serologically positive for dengue fever. Among the 131 patients 60% had DF, 15% DHF grade I, 21% DHF grade II, 4.5% DSS III, and none in DSS IV. IgM positive were 35 (27%), 63 (48%) were positive for both IgG and IgM and 65 (50%) were NS1 Ag positive.

Platelet count of >1,50,000cells / cu.mm was found in 11% of cases and <50,000 in 38% and rest fell in between: Clinical manifestations included are fever (99%), followed by rash (22.13%). Bleeding tendencies were noted in 22% of cases, melena in 8%, hemetemesis 2%; 5% hematuria; 1% ecchymotic patches, 3% hematochezia. Signs of rising hematocrit in 73% patients, evidence of pleural effusion in chest radiograph in 25 patients, ascites in 15 of them, and hypoproteinemia in 10.

Anuradha M, Dendekar RH, Banoo S [72] in their study titled "Laboratory Diagnosis and Incidence of Dengue Virus Infection: A Hospital Based study, Perambalur", done at a teaching hospital in Tamil Nadu screened a total of 151 suspected cases of dengue fever over 1 year (March 2013 to February 2014) by NS1, IgM, IgG ELISA kit out of which 60 (39.74%) cases were seropositive. The peak incidence was found during the month of November followed by December.

Kariyawasam S, Senanayake H [73] in their study titled "Dengue Infections During Pregnancy : Case series from a Tertiary care Hospital in Srilanka", diagnosed and studied a total of 15 pregnant women serology was done by IgM and IgG rapid strip test on serum samples obtained after 5-10 days after the onset of the clinical illness. The age of the patients were between 22 to 41 years. 3 of them were in their second trimester and the rest in their third trimester. IgM only was positive in 6 of them and IgM and IgG (Suggestion of secondary infection) were positive in 9 of the patients. According to the WHO classification, 3 had DF, 3 had DHF grade I, 7 had DHF grade II, 2 developed DSS (1 had DHF II and the other had DHF IV). Fever was present in all the patients (100%), Myalgia (70%). All the patient had thrombocytopenia (100%), Transaminase elevation (AST, ALT alone or both) were seen in all the patients (100%). Platelet transfusion was done in 8 patients (53.3%), 1 patient died of DSS. 2 patients had fetal mortality. Rest of the mothers and children have done well with no evidence of morbidity or malformation seen in any of the children.

Saqib MA, Rafique I, Bashir S, Salam SS [74] in their study quoted "A retrospective Analysis of Dengue Fever case management and frequency of Co-Morbidities associated with deaths" done in 2 major public sector tertiary care hospital during 2011 epidemic of DF in Lahore, Pakistan. A total of 556 who were dengue positive confirmed by IgM, IgG ELISA test were included out of the 390 (70%) were males and rest females. The average age was 36 yrs and the mean duration of stay in hospital was 6 days.439 (78%) were DF, 95 (17%) DHF, 26 (4%) DSS. The mortality among them was 7.2% (40 cases) out of these 17 were diagnosed with DSS. 26 (60%) of the deceased patients had

co-morbid diseases especially hypertension followed by diabetes. DSS was found to be more common in those with essential hypertension. The common clinical findings were fever in 100% of DF, 93% in DHF and 100% in DSS in 75% of DF, 25% in DHF and 92% in DSS; rash in 29% of DF, 25% of DHF, 58% in DSS. Bleeding manifestation in 15% of DF, 59% of DHF and 72% of DSS patients. Thrombocytopenia was observed (<10,0000) was observed in 515 (93%) of patients but severe thrombocytopenia was seen in 29 (5%) of cases (<10,000 cells/cumm) platelet transfusion was done in 260 patients. (192 DF, 48 DHF and 20 DSS) out of which 19 patients had severe thrombocytopenia which 173 cases had platelet count in the range of 11,000 to 40,000 cells/cumm. About 68 (12%) of the patients were given platelet transfusion even with their count >40,000.

Saqib MA, Rafique I, Bashir S, Salam SS [76] in their study titled "Severity of Acute Hepatitis and its outcome in patients with Dengue fever in a Tertiary care Hospital Karachi, Pakistan".According to this study severe hepatitis complicating DF is a major contributor to life threatening haemorrhage, encephalopathy and disseminated intravasular coagulation (DIC). The complications and mortality between patients with mild to moderate (ALT between 23-300 IU/L) versus severe hepatitis (ALT > 300 IU/L) were studied. A total of 699 patients with dengue fever were included. Out of them 605 (87%(and DF, 94 (13%) DHF or DSS. Liver function test (LFT) revealed mean ALT of 88.50 IU/L (range of 43.25 – 188 IU/L) ,AST of 174 IU/L (range of 87-371.5 IU/L) and mean S.bilirubin of 0.8 mg/dl (range of 0.6-1.3mg/DL). Overall mortality was 33.3% in moderate hepatitis group versus 66.7% in severe hepatitis group. There was a considerable difference for complications like

bleeding (P value <0.001), acute renal failure (P value 0.002), acalculus cholecystitis (P.value 0.04) and encephalopathy (P Value 0.02) between mild / moderate to severe hepatitis group the average length of stay in hospital was 3.63 days compared to 4.3 days in those who had mild/moderate hepatitis and compared to patients with severe hepatitis . They concluded that severe hepatitis (SGPT-ALT of >300 IU/L) in DF is associated with prolonged in hospital stay, bleeding, renal failure and mortality.

Pruthvi D, Shashikala P, Shenoy V[78] in their study titled "Valuation of Platelet count in Dengue Fever Along with Seasonal Variation of Dengue infection done at a teaching institution in Davangere, Karnataka, India, screened a total of 1549 patients experiencing febrile illness January 2009 to December 2009 for dengue infection. Out of them 264 (17.04%) cases were confirmed serologically positive for DF by IgM ELISA assay. The peak incidence of DF were noted in the post monsoon time when there is relative drop in temperature as well as a rise in humidity. Platelet counts were <25,000 in 472 cases (30.47%) between 25,000 – 49,000 in 386 cases (24.91%), between 50,000 – 74,000 in 376 case (24.27), between 75,000 – 1,00,000 in 169 cases (10.49%) and >1,00,000 in 146 cases (9.42%).

Khan MU, Rehman R, Gulfraz M, Latif Wet al[80] in their study quoted "Incidence of Thrombocytopenia in Seropositive Dengue patients" at University of Health Sciences, Lahore, Pakistan. Samples were taken and screened from 750 patients who presented with febrile illness consistent with dengue fever from September 2011 to January 2012 with IgM ELISA serology. A total of 250 patients were confirmed positive for DF. 1% of patients had mild to moderate hepatitis and 103 (15%) had severe hepatitis Among the 250 patients 2% had

severe thrombocytopenia, 65.2% had mild to moderate thrombocytopenia and 32.8% had normal platelet counts (82 patients had normal platelet count), 5 patients had severely low count i.e. < 25,000/cu.mm, 106 patients had moderately low counts i.e. between 25,000 – 1,00,000 cells/ cu.mm and 57 patients had borderline reduction ie) between 1,00,000 to 1,50,000 cells/cu.mm. Bleeding manifestation was absent in normal and borderline thrombocytopenia groups (0%), present in moderately low (23%) and severely low (80%) groups. Bleeding manifestation and thrombocytopenia revealed no gender or age differences bleeding risk was significantly related to thrombocytopenia.

Khan M, Kuppusamy K, SumathiS et al[83] in their study titled "Evaluation of Thrombocytopenia in Dengue infection along with seasonal variation in Rural Melmaruvathur" conducted at a teaching hospital in Melmaruvathur, Tamil Nadu, India between January 2011 to December 2012 screened a total of 1,464 patients presented execute febrile illness consistent with dengue fever using dengue IgM ELISA serology test out of the 1464 patients, 107 (7.3%) tested positive for DF, 76 (71%) of the positive cases had thrombocytopenia (<1,00,000 cells / cu.mm) while the remaining 31 (19%) had platelet count of >1,00,000 cells/cu.mm. Out of the 76 patients with thrombocytopenia 38 (50%) had mild thrombocytopenia (5,1000-1,00,000) 28 (36.8%) had moderate thrombocytopenia (20000-50000) and the rest 10 (13.2%) had severe thrombocytopenia (<20,000). On admission of the 107 seropositive cases, 58 (54.2%) had DF, 38 (35.5%) had DHF and 11 (10.2%) had DSS. Thrombocytopenia was more pronounced in patients with DHF/DSS.

Among the patients with thrombocytopenia 47.4% cases with mild thrombocytopenia, 42.3% with moderate thrombocytopenia and 100% with

severe thrombocytopenia presented with clinical features suggestive of DHF/DSS. The incidence of seropositivity was predominantly seen during the post monsoon and monsoon period ie). premonsoon 15 cases (14%), monsoon 31 cases (29%), and post monsoon 61 cases (57%). Follow up of cases revealed all cases of DF, and 31 case of DHF had complete recovery and remaining 18 cases (7 DHF & 11 DSS) were referred to a higher specialized centre for further management.

Harrisons Text Book of Internal medicine states that an isolated platelet count of <50,000/µl is associated with minor risk of bleeding until the count drops below <10,000-20,000/µl. There is increased risk by bleeding when associated with coexisting coagulopathies, liver disease, platelet inhibiting drugs and infection. The minimum threshold for platelet transfusion is <10,000/µl in otherwise stable patients.[7]

Kulkarni N[84], in her study of 232 patients of dengue at a medical college hospital in Karnataka during July 2011 to October 2011 , out of this 195 (84.2%), 35 (15%), 02(0.8%) were DF, DHF, DSS respectively. 118(51%) cases received single unit of platelet transfusion between 20,000-1,000,00/µL and 64 (27.5%) patients who had platelet count of less than 20,000/µl received multiple platelet transfusion. It was concluded that 51% of platelet transfusion are inappropriate and is more effective when it is given to patient with platelet count of <20,000µl.

The present study was conducted in the Department of Pathology, Sree Mookambika Institute of Medical Sciences, Kulasekharam, Kanyakumari District, Tamil Nadu. In the present study the cases were taken from the Department of General Medicine, Sree Mookambika Institute of Medical Sciences, Kulasekharam. Clinically suspected cases of dengue confirmed by serological studies of IgM ELISA with or without NS1 antigen positivity were taken and the various parameters were studied. They were followed from the day of admission to time of recovery or discharge.

MATERIALS AND METHODS:

a) **Study design:** Descriptive Cross Sectional Study
b) **Study setting :**SreeMookambika Institute of Medical Sciences
c) **Approximate total duration of the study:** Two years(till sample size was reached)
d) **Number of groups studied:** One
e) **Detailed description of the groups:** Patients in the age group 13 and above, attending the Medicine OPD of SMIMS, Kulasekharam, considering the inclusion and exclusion criteria.

i) Inclusion criteria:

1. All serologically positive dengue patients (Dengue IgM antibody,NS1 antigen)
2. Age 13 years and above.

ii) Exclusion criteria:

1. Suspected dengue cases in which serology is found to be negative.
2. Serologically positive cases of dengue who are also positive for other coexisting infections eg. Malaria, typhoid.
3. Patients not giving consent.

Statistical method of analysis:

Mean, Standard deviation and Statistical parameters are calculated by utilizing statistical analysis package. Suitable tests of analysis are analysed

Method(s)/Technique(s)/Instrument(s)/Reagent(s)/Kit(s) etc used to measure the quantitative parameters along with the manufacturing source details:

NS1/IgM ELISA KIT: Manufacturer: Panbio, Inverness Medical Innovations, (Australia Pty Ltd,532 seventeen Miles Rocks Rd, Sinnamon Park, QLD 4073 Australia)

Leishman's stain: Manufacturer: Biolab diagnostics,Cochin-25,Cochin.Phone No:9388619462

Automated cell counter: Beckman Coulter Ac.T 5 Diff CP: Manufacturer: System Technologies, LLC, New Hope, MN, Phone number:763-537-3600.

T- COAG KC1 DELTA: Trinity Biotech, IDA business park, Co Wicklow, Ireland. Tel:+353 1 276 9800

DENGUE NS1 CAPTURE ELISA:

Principle

Serum dengue NSI antigen when present binds to anti-NS1 antibodies present on the polystyrene surface of microwells. Residual serum is removed by washing and HRQ conjugated Anti NS1 MAb is supplemented. After incubation, the microwells are washed and a colourless substrate system, tetramethylbenzidine / hydrogen peroxide (TMB chromogen) is then added. The substrate is hydrolysed by the enzyme and the TMB becomes a blue colour. After stopping the reaction with acid, the TMB turns yellow. Development of colour is suggestive of the presence of dengue NS1 antigen in the test sample.

Materials

1. Anti-NS1 Antibody coated microwells (12x8wells)
2. HRP conjugated Anti-NS1 MAb – 1 bottle 15 ml
3. Wash Buffer (20x) – One bottle of 20x concentrate of phosphate buffered saline (pH – 7.2-7.6) with Tween 20 and preservative (0.1% Proclin)
4. Sample Diluent – one bottle, 22ml Brown
5. TMB Chromogen (TMB) – one bottle 15ml
6. Positive control – one purple – capped vial, 1.2ml of recombinant antigen
7. Calibrator – Two orange – capped vials, 1.5ml
8. Negative control – one white capped vial, 1.2ml of human serum.
9. Stop solution – one Red – capped bottle, 15ml.

Additional materials required but not provided.

1. Accurate adjustable micropipetters which have disposable pipette tips (5 – 100µl)
2. Deionised water.
3. Microplate washing system
4. Microplate reader with 450nm filter
5. Timer
6. Graduated cylinder
7. Flask
8. Test tubes / microplate for dilutions.

Storage conditions

All reagents/materials are stored at 4^{o}C.

Specimen to be used

The test has to be done on serum only. The use of whole blood, plasma or any other specimen matrix has not been recognized.

ELISA PROCEDURE

i) Pipette 100µL diluted test sample and controls onto their respective microwells.

ii) Cover the plate and incubate for 1hr at 37^{o}C ± 1^{o}C

iii) Wash six times with diluted wash buffer.

iv) Pipette 100µL HRP conjugated Anti NS1 MAb into each well.

v) Cover plate and incubate for 1 hr at 37^{o}C ± 1^{o}C

vi) Wash six times with diluted wash Buffer.

vii) Pipette 100µL of TMB into each well.

viii) Incubate for 10 minutes at room temperature (20 – 25^{o}C), timing from the first addition. A blue colour will develop.

ix) Pipette 100µL of stop solution to all wells in the same sequence and timing as the TMB addition. Mix well. The blue colour changes to yellow.

x) In 30 minutes read the absorbance of each well at a wavelength of 450nm with a reference filter of 600-650nm.

Quality Control

Each kit is supplied with one positive control and one Negative control and a calibrator.

Interpretation of results

Index	Panbio Units	Result
< 0.9	< 9	Negative
0.9 – 1.1	09 -11	Equivocal
>1.1	>11	Positive

DENGUE IgM CAPTURE ELISA:

Principle:

The panbio ELISA kit contains a microtitre plate, which is precoated with dengue virus antigens. At the time of the first incubation, anti dengue IgM antibodies in the diluted patients serum gets bound to dengue virus antigens. Following this incubation all the unbound material are removed by washing using an ELISA washer. The presence of antidengue IgM antibody is detected by adding and incubating peroxidase enzyme labeled antihuman IgM. This is followed by a washing step. The enzyme activity can this be detected by adding chromogen/substrate solution (ie) Tetra Methyl Benzedine. The absorbance is read at 540nm using ELISA microwell plate reader.

The test was carried out the results were calculated according to the manufacturers instructions in the insert.

Content

- Coated microplate wells
- Positive control (IgM, human)
- Negative control (IgM, human)
- Calibrator
- Enzyme conjugate
- Sample buffer (has IgG/Rheumatoid factor (RF) – absorbent
- Wash buffer
- Chromogen/substrate (TMB/H_2O_2)
- Stop solutions (0.5ml, sulphuric acid)

Preparation of Reagents

Preparation of wash buffer:

It has a strength of 10x, it was prepared by adding 1 part of reagent with 9 part of deionized or distilled water.

Preparation of patients sample

Patients serum sample has to be diluted using sample buffer in a ratio of 1 = 101 before analysis. For this 10µl of serum was added to 1.0ml of sample buffer and mixed well. The sample buffer contains anti-human IgG antibody from goat. The human IgG antibodies in the serum binds to the above antibodies and precipitate leaving only IgM antibodies. This was allowed to incubate at room temperature for 10 minutes. This step was done to remove IgG antibody and rheumatoid factors.

Procedure of ELISA

1. The reagents are brought to room temperature (+18 to +25^{o}C) before the commencement of the assay.
2. Patients serum samples are processed as mentioned earlier in the preparation of patients samples.
3. 100µl of calibrator, negative control , positive control and diluted patients samples are pipetted into individual microplate wells in accordance to the pipetting protocol.
4. Incubated at room temp (+18^{o}c to 25^{o}C) for 30 minutes
5. Wash the reagent wells 3 times with 400µl working strength wash buffer. Leave the buffer in each well for 30 – 60 sec per washing cycle. After washing to remove all liquid from microplate by tapping an absorbant paper with opening facing downwards.
6. Pipette 100µl of enzyme conjugated (peroxidase labeled antihuman IgM) into each microplate well.
7. Incubate at room temperature (+18^{o} to 25^{o}C) for 30 minutes.
8. Wash as above
9. Pipette 100µl of chromogen /substrate solution to each of the microtitre well.
10. Incubate at room temperature (+18^{o}C to 25^{o}C) for 15 minutes (protect from direct sunlight)
11. Pipette 100µl of stop solution into each of the microplate wells.
12. Read the absorbance of wells with a bichromatic spectrophotometer at 450nm wavelength and reference wavelength between 620nm and 650nm within 30 minutes of addition of the stop solution.

Calculation of Results

The extinction value of calibrator defines the upper limit of the reference range of non infected persons (cut offs). Values above the indicated cut off are measured as positive, those below are considered negative.

Semi Quantitative

Results can be concluded semi quantitatively by calculating ratio of extinction value of the control or patient sample over the extinction value of calibrator.

$$\text{Ratio} = \frac{\text{Extinction of control or patient sample}}{\text{Extinction of calibrator}}$$

Interpretation :

Ratio < 1.8 - negative

Ratio ≥ 1.8 to 22 – borderline

Ratio ≥ 2.2 – positive

Additional materials required but not provided

1. Accurate adjustable micropipettors with disposable pipette tips
2. Microplate washing system
3. Deinoised water
4. Microplate reader with 450nm filter
5. Graduated cylinder
6. Timer
7. Test tubes or microtitre plate for serum dilutions
8. Flask

ESTIMATION OF aPTT PT- INR:

Principle:

Tissue thromboplastin in the existence of calcium activates the extrinsic pathway of the human blood coagulation system. When LIQUIPLASTIN reagent is added to normal anticoagulated plasma, the clotting mechanism is activated forming a solid gel clot within a specific period of time. The time essential for clot formation would be protracted if there is a deficiency of factors/factor activity in the extrinsic pathway of coagulation system.

Test procedure:

1) Aspirate from the reagent vial for instantaneous testing requirements in a clean and dry test tube.
2) To a 12x75mm tube add 0.1 ml of plasma
3) To the tube place a steel ball and with force add 0.2 ml of LIQUIPLASTIN reagent and simultaneously press start and the tube is automatically rotated.
4) Stop the machine as soon as the first fibrin strand is visible and the gel/clot formation begins
5) Time is recorded in seconds. This gives the prothrombin time, aPTT values are also noted.
6) INR is calculated from the PT ratio using a thromboplastin with a known ISI.

ESTIMATION OF HAEMATOLOGICAL PARAMETERS:

Estimation of haematological parameters like Hb%,PCV,WBC count, platelet count, differential count were done by automated cell counter (Beckman Coulter Ac.T 5 Diff).

PERIPHERAL SMEAR STAINING AND EXAMINATION:

Blood smears are prepared from EDTA blood and are stained by Leishman stain

The components of Leishman stain are:

1. Methylene blue
2. Eosin
3. Absolute methyl alcohol

Procedure:

- Air dry the smear and cover the smear with Leishman stain for two minutes
- After two minutes add twice the volume of buffered water and leave for 5-7 minutes.
- A scum of metallic sheen forms on the surface.
- Wash the stain away in tap water
- Wipe the back of the slide clean and set it upright on a draining rack to dry.
- Mount the slide with DPX with a clean and dry 25x25mm cover slip
- The slide is then examined.

Source of data:

The present study is carried out in 50 cases of serologically positive dengue patients in the Department of Pathology, Sree Mookambika Institute of Medical Sciences, Kulasekharam, TamilNadu.

Method of collection of Data:

- The study is done in collaboration with the Department of Medicine.
- Clinically suspected cases of dengue will be screened and their consent for the study obtained.
- Patients clinical data were collected from the medical records.
- Data was then be collected in a predesigned questionnaire.
- 2-5ml of venous blood is collected in vaccum tubes or disinfected vials with screw caps and gasket.
- Adhesive tape marked with pencil, permanent ink or a type written/printed self adhesive label to recognize the container. The name of the patient, identification number and date of collection will be indicated on the label
- Serological confirmation by dengue specific NS1 antigen assay and/or IgM by ELISA was done. In this method the plate is sensitized with the antigen. It is then washed and the test antibody is added. Another wash is given and a chromogen is added. The results are quantitated by colorimetric scanning of the plate.

- Clinical parameters of each serologically dengue positive patient was recorded.
- Other haematological parameters like Haemoglobin, Packed cell volume, Total WBC count, Differential WBC count was done by automated cell counter and recorded.
- Platelet count was done by automated cell counter . If flagging occurs for the values manual count will be done.
- Peripheral smears were made and stained with Leishman's stain and the report of the smear was recorded.
- Biochemical parameters like AST, ALT, aPTT, PT,S. Bilirubin,, BUN,S. Creatinine, and Blood glucose values was recorded.
- An observation on the number of dengue positive patients who require and receive platelet transfusion as per the treatment norms for dengue patients in the Medicine department of SMIMS was made and the details of the transfusion noted.
- The patients who were observed to receive platelet transfusion were followed up with repeated platelet counts (for which 2ml of venous blood will be drawn) and results of the platelet count and outcome noted.

The data obtained was tabulated in the master chart with the various parameters to be observed and studied.

Age distribution:

Out of fifty cases of serologically positive dengue cases studied 8(16%) cases were in the age group of 14-20 years,13(26%) were in the age group 21-30 years,13(26%) were in the age group 31-40 years,7(14%) were in the age group of 41-50 years,3(6%) were in the age group of 51-60 years,5(10%) were in the age group of 61-70 years and one (2%) case was above 70 years of age.(Table 1,Figure 1)

In my study the commonest age group was from the age of 14-40 years.

Gender distribution:

Out of the 50 cases studied 29(58%) were males and 21(42%) were females.(Table 2 ,Figure 2)

In my study there was a slight male preponderance.

Fever duration distribution:

Out of the fifty cases studied patients who had fever for 5 days duration were 13(26%),fever for 6 days were 17(34%),fever for 7 days were 12(24%),fever for 8 days were 3(6%),fever for 9 days were 1(2%) and fever for 10 days duration was 4(8%).(Table 3,Figure 3)

5-6 days was the most common fever duration in my study.

Musculoskeletal complaints distribution:

Out of the fifty cases studied 50(100%) of the patients had headache, 43(86%) had myalgia ,7(14%) had retroorbital pain, and 5(10%) had arthralgia.(Table 4)

Respiratory complaints distribution:

Out of the fifty cases studied 6(12%) of the patients had upper respiratory tract infection, 8(16%) had lower respiratory tract infection.(Table 5)

Gastrointestinal complaints distribution:

Out of the fifty patients,14(28%) had abdominal pain,20(40%) had vomiting,26(52%) had hepatomegaly and 24(48%) had splenomegaly.(Table 6)

Haemorrhagic manifestation distribution:

Out of the fifty cases studied only one(2%) of the patients had major bleeding manifestation.8(16%) of patients had minor bleeding manifestations and 41(82%) had no bleeding manifestation.(Table 7,Figure 4)

Signs of capillary leak distribution:

Out of the fifty cases studied,8(16%) of cases had pleural effusion,13(26%) had ascitis and 8(16%) had rising haematocrit.(Table 8)

Serological positivity distribution:

Out of the fifty cases studied 29(58%) showed positivity for IgM and 21(42%) showed positivity for NS1 and IgM.(Table 9,Graph 5)

Mean values of haematological examination:

In the fifty cases studied the mean values of haemoglobin was 12.78 with a S.D value of 1.71,PCV was 36.25 with a S.D of 6.28,WBC count was 4998.80 with a S.D value of 2.52.The platelet count mean was 25220.74 with a S.D value of 4.62.The mean value for aPTT was 37.18 with a S.D value of 1.07.The PT INR mean value was 1.14 with a S.D value of 0.44.(Table 10,Graph 6)

Mean values of differential count:

In the fifty cases studied the mean values for polymorphs were 52.32 with S.D value of 1.30,mean value for lymphocytes was 30.52 and S.D of 1.16,mean value of eosinophils was 2.08 and S.D of 2.58.The mean value for monocytes is 14.07 and S.D is 10.28 and mean value for basophils was 1.69 with S.D value of 1.83(Table 11,Graph 7)

Peripheral smear examination distribution:

In the fifty patients studied 4(8%) had anemia,3(6%) had leukopenia,16(32%) had thrombocytopenia,18(36%) had leukopenia with thrombocytopenia,8(16%) had anemia with thrombocytopenia and 6(12%) of the patients had anemia with leukopenia and thrombocytopenia.(Table 12)

Distribution of patients according to grade of thrombocytopenia:

In the 50 patients studied 48 patients show thrombocytopenia .12(25%) showed Grade 1 thrombocytopenia,5(10.42%) Grade 2,22(45.83%) Grade 3 and 9(18.75%) Grade 4.(Table 13,Graph 8)

WBC count distribution:

In the fifty cases studied Leukopenia was observed in 22(73%) of the patients,Leukopenia with reversal of neutrophil lymphocyte ratio was observed in 6(20%) of the patients,2(7%) had only reversal of neutrophil lymphocyte ratio.(Table 14,Graph 9)

Mean values of biochemical examination:

The various biochemical parameters observed in this study,blood glucose with a mean of 141.60 and S.D of 7.71,AST mean value of 106.08 with a S.D of 8.96,ALT mean value of 118.54 and S.D of 9.63,total bilirubin mean value of 3.47 and S.D of 1.46,urea mean value 32.70 with S.D of 1.95 and mean value for creatinine 1.15 with S.D 1.12.(Table 15)

Distribution of patients administered platelet transfusion according to indication :

In this study among fifty patients the patients where platelet transfusion was indicated were 43(86%).Thrombocytopenia was observed in 48(96%).(Table 16)

Distribution of patients according to initiation of platelet transfusion:

In this study 2(4%) patients had platelet transfusion on the day of admission,26(52%) on the first day after admission,8(26%) on the second day after admission and 7(14%) on the third day after admission.(Table 17)

Distribution of patients according to units of platelets transfused:

In this study 27(54%) of the patients were infused 1-2 units of platelets,5(10%) of patients were given 3-4 units of platelets,8(16%) of patients were given 5-6 units,2(4%) were given 7-8 units of platelets and 1(2%) was given 9-10 units.(Table 18,Graph 10)

Distribution of patients according to the number of days of return to normal platelet count:

In this study of 50 patients,43 were transfused platelets 3(6.98%) took 2 days for platelet count to return to normal levels,23(53.49%) took 3 days,9(20.93%) took 4 days,3(6.98%) took 5 days and 5(11.63%) took 6 days.(Table 19,Graph 11)

Distribution of patients according to diagnosis:

In this study of fifty patients 29(58%) had dengue fever and 21(42%) were diagnosed as DHF.(Table 20,Graph 12)

Distribution of patients according to recovery from dengue fever:

In the fifty patients studied 4(8%) of patients took less than 7 days to recover from dengue fever,45(90%) took 7-13 days to recover and 1(2%) took 14 days.(Table 21,Graph 13)

STATISTICAL ANALYSIS

Table-1: Distribution of patients according to age

Age (years)	Number	Percentage (%)
14-20	08	16.00
21-30	13	26.00
31-40	13	26.00
41-50	07	14.00
51-60	03	06.00
61-70	05	10.00
Above 70	01	02.00

Age group ranging from 21-40 showed the maximum distribution

Table-2: Distribution of patients according to gender

Gender	Number	Percentage (%)
Male	29	58.00
Female	21	42.00

Males were more affected than females

Table-3: Distribution of patients according to duration of fever

Duration of fever	Number	Percentage (%)
5 days	13	26.00
6 days	17	34.00
7 days	12	24.00
8 days	03	06.00
9 days	01	02.00
10 days	04	08.00

Days 5-6 showed the maximum percentage.

Table-4: Distribution of patients according to musculoskeletal complaints

Musculoskeletal complaints	Number	Percentage (%)
Headache	50	100
Retro orbital pain	07	14.00
Myalgia	43	86.00
Arthralgia	05	10.00

Headache was seen in 100% of patients followed by myalgia.

Table-5: Distribution of patients according to respiratory complaints

Respiratory complaints	Number	Percentage (%)
Upper respiratory tract infection	06	12.00
Lower respiratory tract infection	08	16.00
Dyspnoea	00	00.00

More number of people (16%) were affected with lower respiratory tract infection.

Table-6: Distribution of patients according to gastrointestinal complaints

Gastrointestinal complaints	Number	Percentage (%)
Abdominal pain	14	28.00
Vomiting	20	40.00
Diarrhoea	07	14.00
Hepatomegaly	26	52.00
Splenomegaly	24	48.00

Hepatomegaly was seen in 52% of patients

Table-7: Distribution of patients according to hemorrhagic manifestation

Hemorrhagic manifestation	Number	Percentage (%)
Major	1	02.00
Minor	8	16.00
Absent	41	82.00

16% showed minor bleeding manifestations like petichiae and bleeding gums

Table-8: Distribution of patients according to signs of capillary leak

Signs of capillary leak	Number	Percentage (%)
Pleural effusion	8	16.00
Ascites	13	26.00
Rise of hematocrit	8	16.00

Rise in haematocrit is seen in 16% of the patients,Ascites in 26%.

Table-9: Distribution of patients according to serological examination

Serology examination	Number	Percentage (%)
NS1(Nonstructural protein -1)	0	00.00
IgM (Immunoglobulin M)	29	58.00
NS1+IgM	21	42.00

IgM positivity was seen in 58% of cases,NS1 and IgM positivity seen in 42% of cases.

Table-10: Mean values of hematological examination

Hematological examination	(MEAN±SD)
Hb%(gm/dl)	12.78±1.71
PCV(L/L)	36.25±6.28
WBC(cells/cu.mm)	4998.80±2.52
Platelet count(cells/cu.mm)	25220.74±4.62
APTT(seconds)	37.18±1.07
PT INR(ratio)	1.14±0.44

Mean platelet count was 25,220.74

Table-11: Mean values of differential count

Differential count (%)	(MEAN±SD)
Polymorphs	52.32±1.30
Lymphocytes	30.52±1.16
Eosinophils	02.08±2.58
Monocytes	14.07±10.28
Basophils	01.69±1.83

Mean DC values were Polymorphs(52.32%),Lymphocytes(30.52%).

Table-12: Distribution of patients according to peripheral smear examination

Peripheral smear examination	Number	Percentage (%)
Anemia	04	08.00
Leukopenia	03	06.00
Thrombocytopenia	16	32.00
Leukopenia with Thrombocytopenia	18	36.00
Anemia with Thrombocytopenia	08	16.00
Anemia+ Leukopenia + Thrombocytopenia	06	12.00

Thrombocytopenia was seen in 96% of cases

Table-13: Distribution of patients according to grade of thrombocytopenia

Grade of thrombocytopenia	Number	Percentage (%)
Grade-1 (75,000 to 1,50,000) cells/cu.mm	12	25.00
Grade-2 (50,000 to less than 75,000) cells/cu.mm	5	10.42
Grade-3 (25,000 to less than 50,000) cells/cu.mm	22	45.83
Grade-4 (less than 25,000) cells/cu.mm	9	18.75

Grade 3 showed 45.83% of cases

Table 14: Distribution of patients according to WBC count and distribution

WBC count and distribution	Number	Percentage (%)
Leukopenia (<4000 cells/cu.mm)	22	73.00
Leukopenia with reversal of neutrophil lymphocyte ratio	6	20.00
Reversal of neutrophil lymphocyte ratio	2	07.00

Leukopenia was seen in93% of cases and 27% showed reversal of neutrophil lymphocyte ratio.

Table-15: Mean values of biochemical examination

Biochemical examination	(MEAN±SD)
Blood glucose(mg/dl)	141.60±7.71
AST(IU/L)	106.08±8.96
ALT(IU/L)	118.54±9.63
Total Bilirubin(mg/dl)	03.47±1.46
Urea(mg/dl)	32.70±1.95
Creatinine (mg/dl)	01.15±1.12

Mean AST levels were 106.08 IU/L and ALT levels were 118.54IU/L

Table-16: Distribution of patients administered platelet transfusion according to indication

According to indication of platelet transfusion	Number	Percentage (%)
Indicated	43	86.00
Thrombocytopenia	48	96.00

86% of the patients were transfused platelets.

Table-17: Distribution of patients according to day of initiation of platelet transfusion

Day of initiation of platelet transfusion	Number	Percentage (%)
0 day	02	04.00
1st day	26	52.00
2nd day	08	16.00
3rd day	07	14.00

Most of the patients were transfused during first or second day

Table-18: Distribution of patients according to units of platelet transfused

Units of platelet transfusion	Number	Percentage (%)
1-2 units	27	54.00
3-4 units	05	10.00
5-6 units	08	16.00
7-8 units	02	04.00
9-10 units	01	02.00

54% of the cases received 1-2 units of platelets

Table-19: Distribution of patients according to the number of days to return to normal platelet count

Number of days	Number	Percentage (%)
2 days	03	6.98
3 days	23	53.49
4 days	09	20.93
5 days	03	6.98
6 days	05	11.63

Most patients had return of platelet count to normal levels by the 3rd or 4th day

Table-20: Distribution of patients according to diagnosis

Diagnosis	Number	Percentage (%)
Dengue fever	29	58
Dengue hemorrhagic fever	21	42

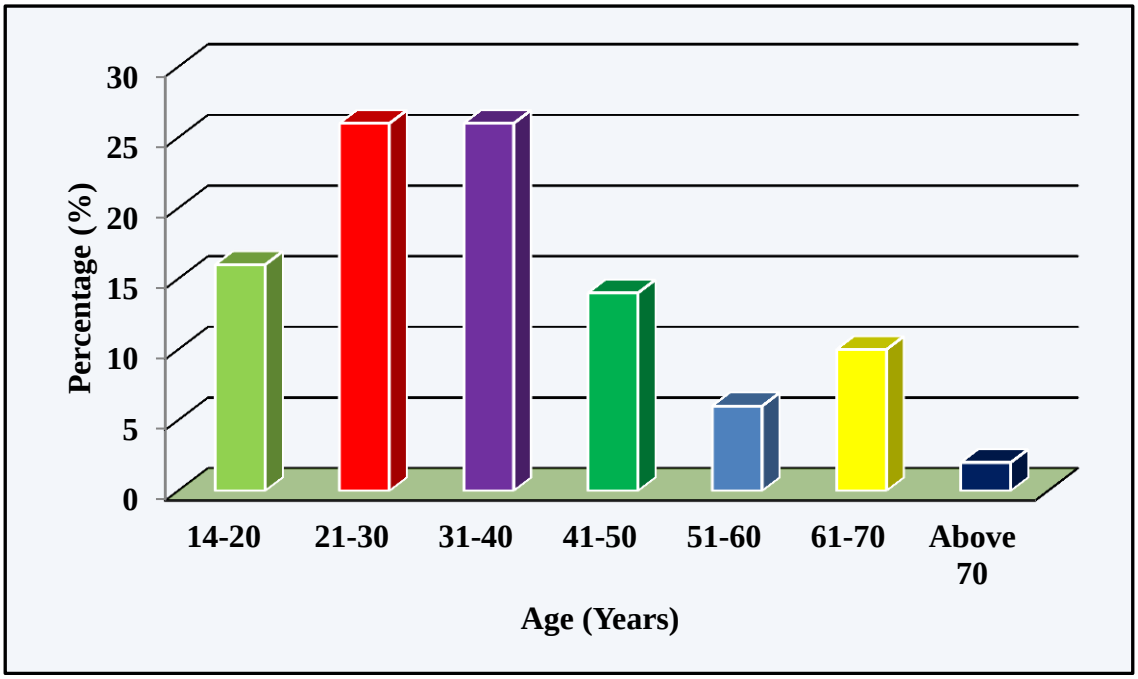
58% of the patients were diagnosed as dengue fever and 42% of the cases as dengue haemorrhagic fever.

Table-21: Distribution of patients according to recovery from dengue fever

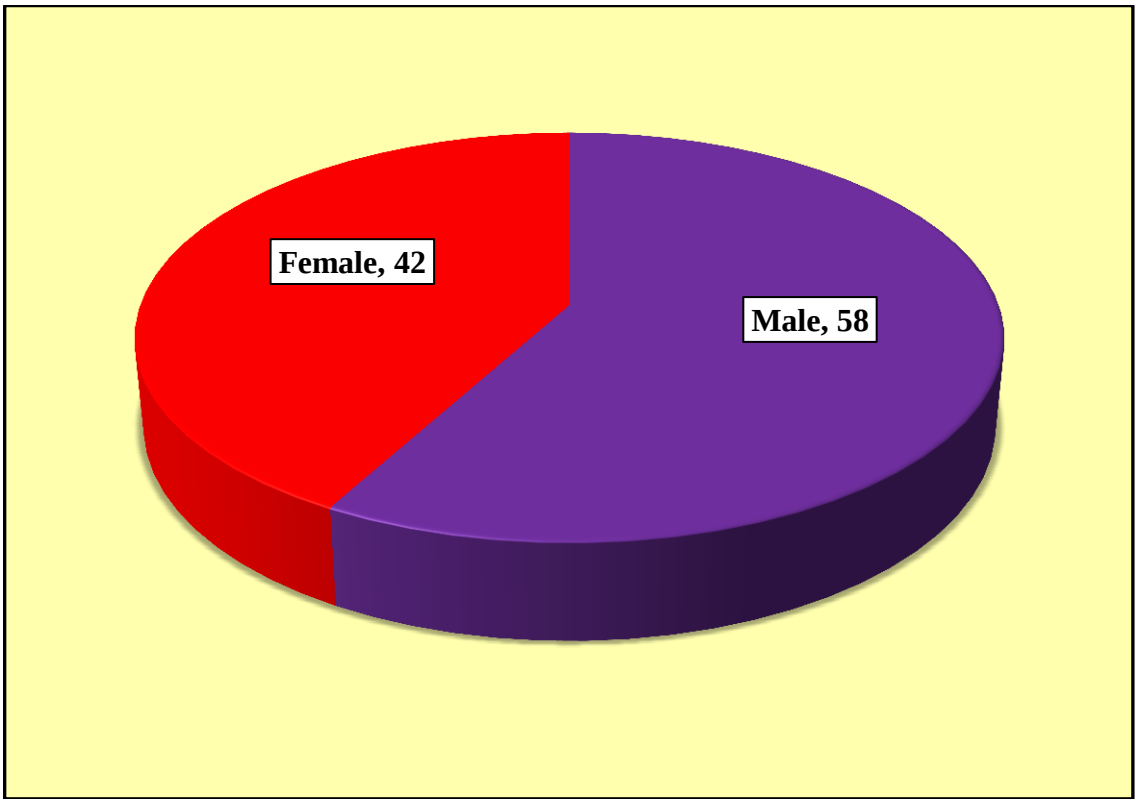
Number of days	Number	Percentage (%)
Less than 7 days	04	08.00
7-13 days	45	90.00
14 days	01	02.00

90% of the cases recovered within 7-13 days

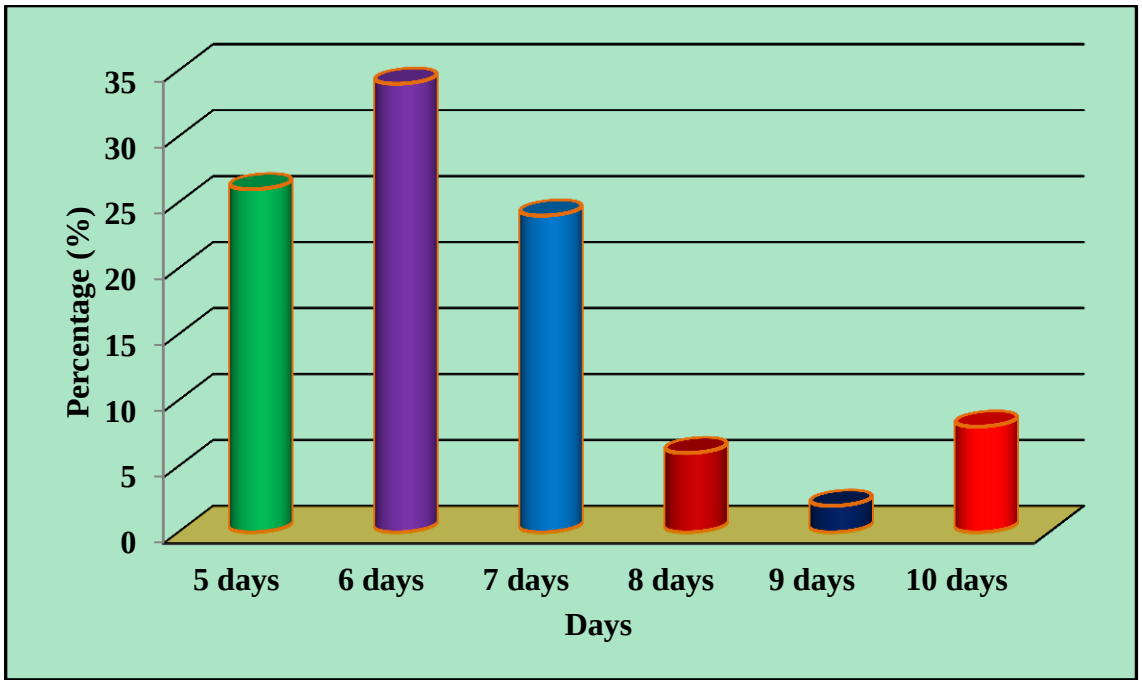
Graph-1: Percentage of patient's distributed according to age



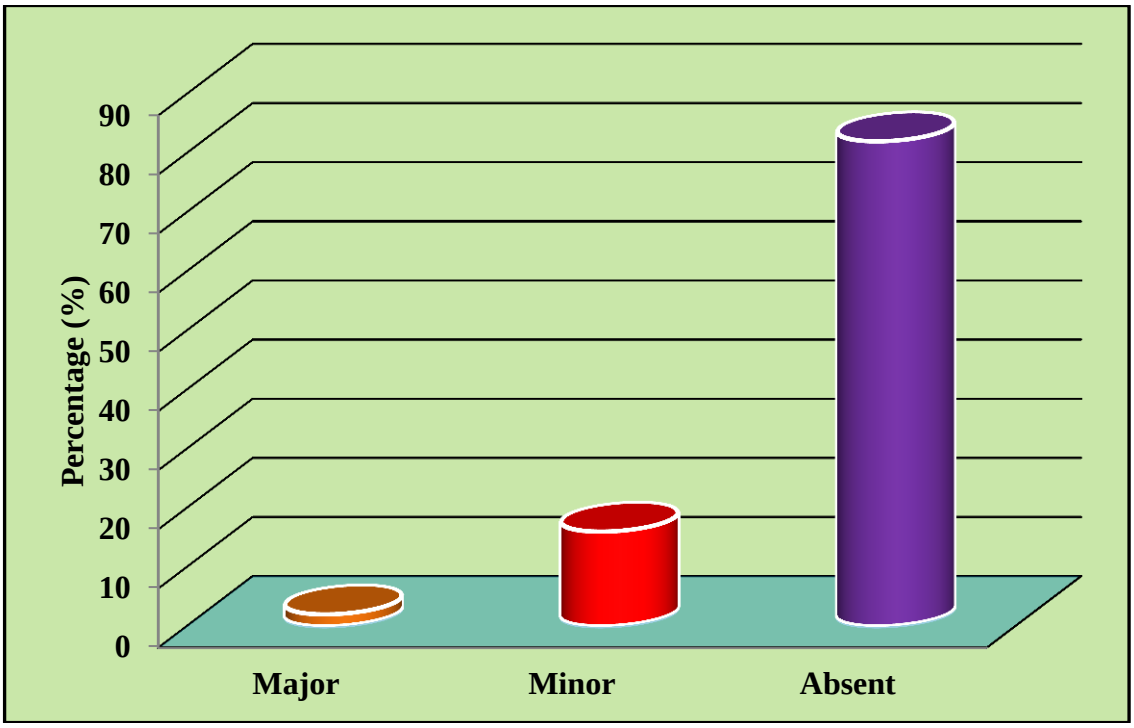
Graph-2: Distribution of percentage of patients according to gender



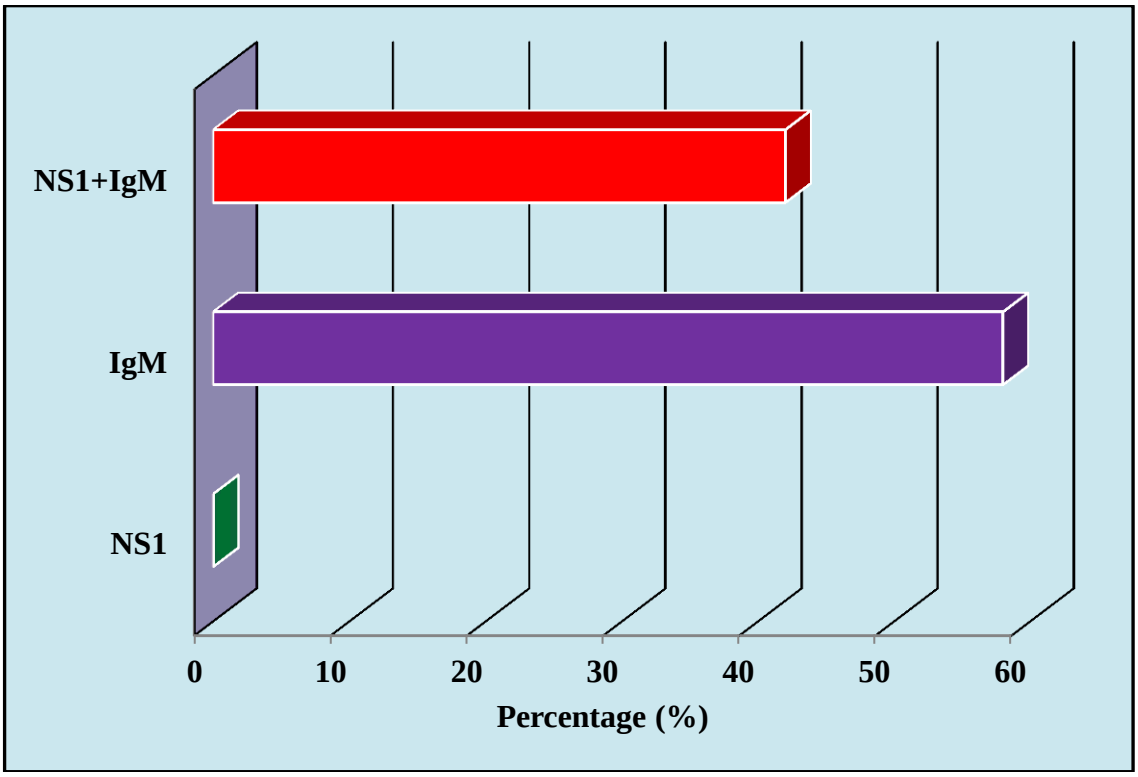
Graph-3: Distribution of percentage of patients according to duration of fever



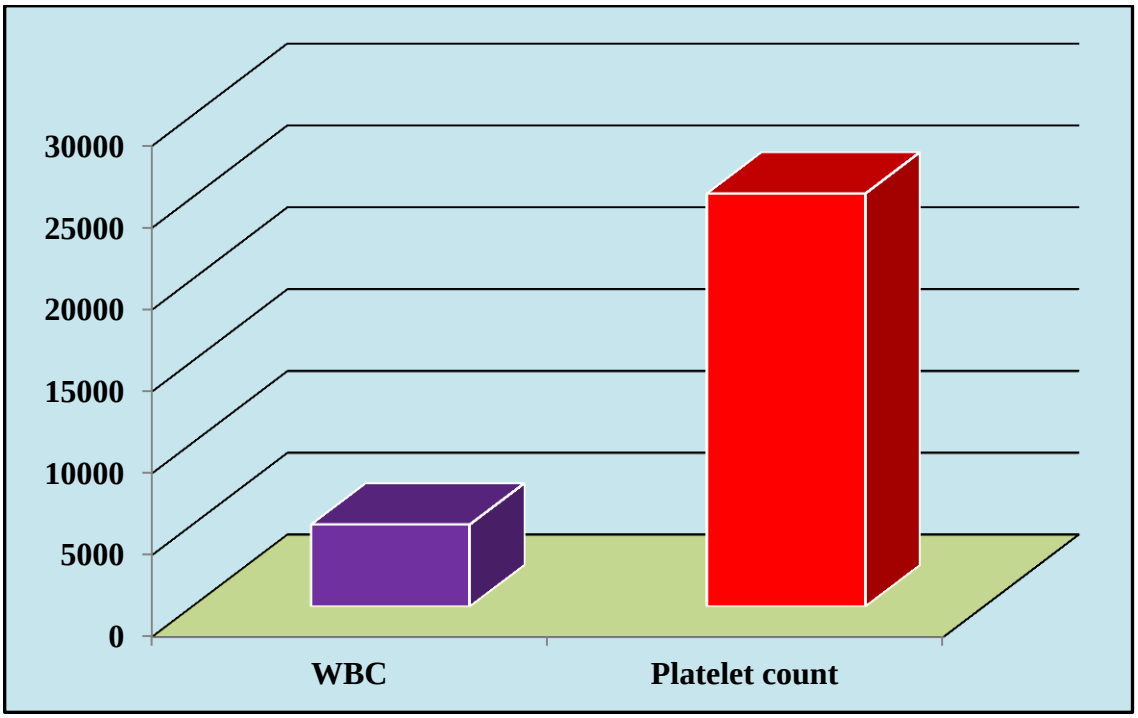
Graph-4: Distribution of percentage of patients according to hemorrhagic manifestation



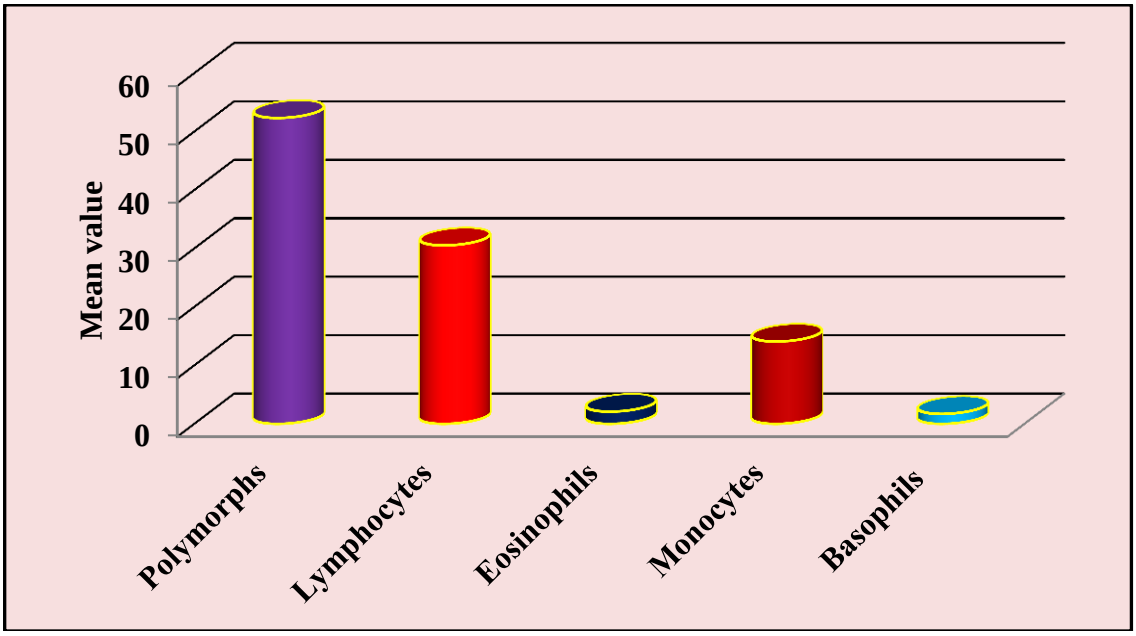
Graph-5: Distribution of percentage of patients according to serological examination



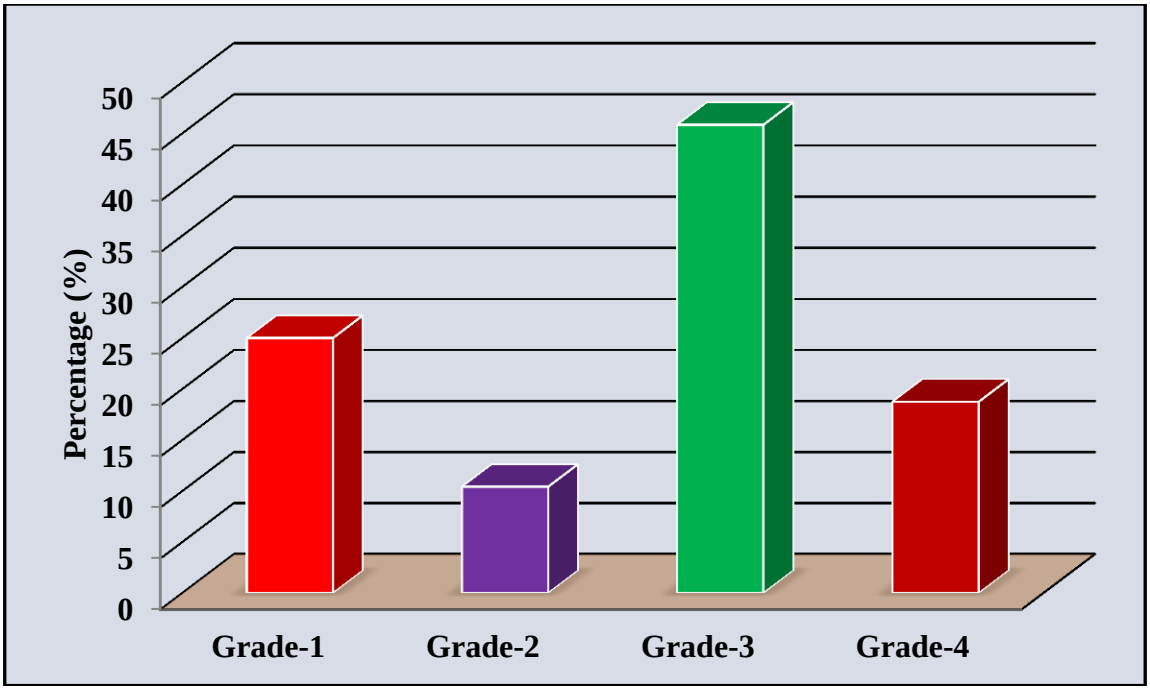
Graph-6: Mean value of WBC and platelet count



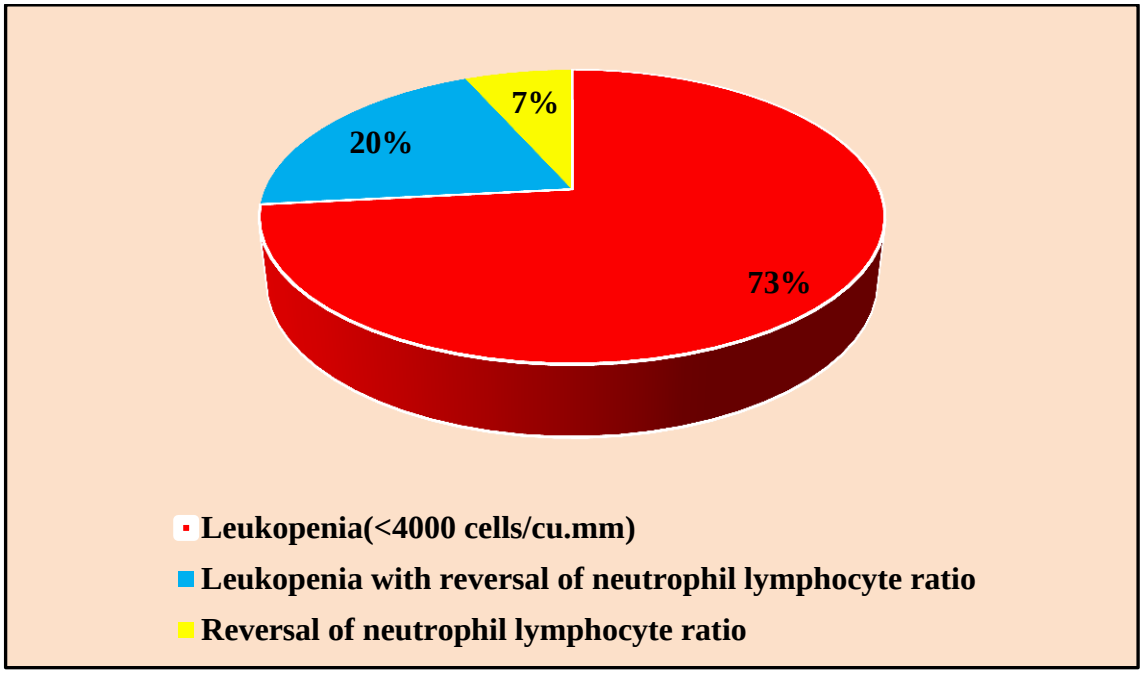
Graph-7: Mean values of differential count



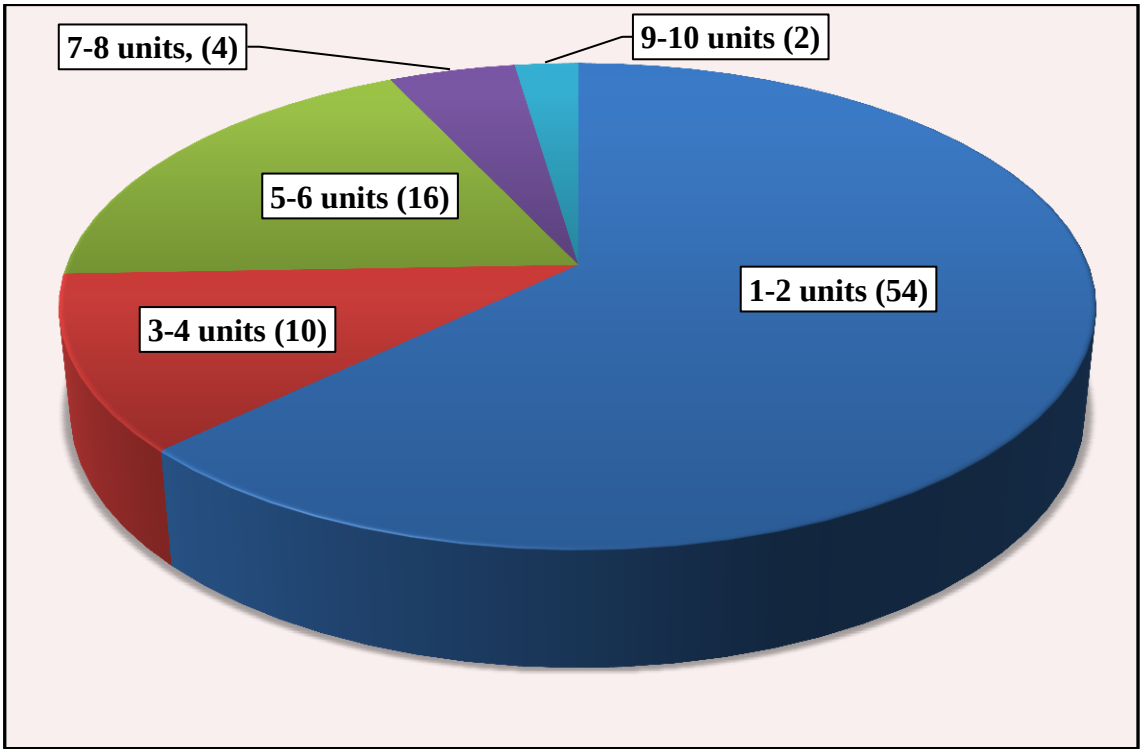
Graph-8: Distribution of percentage of patients according to grade of thrombocytopenia



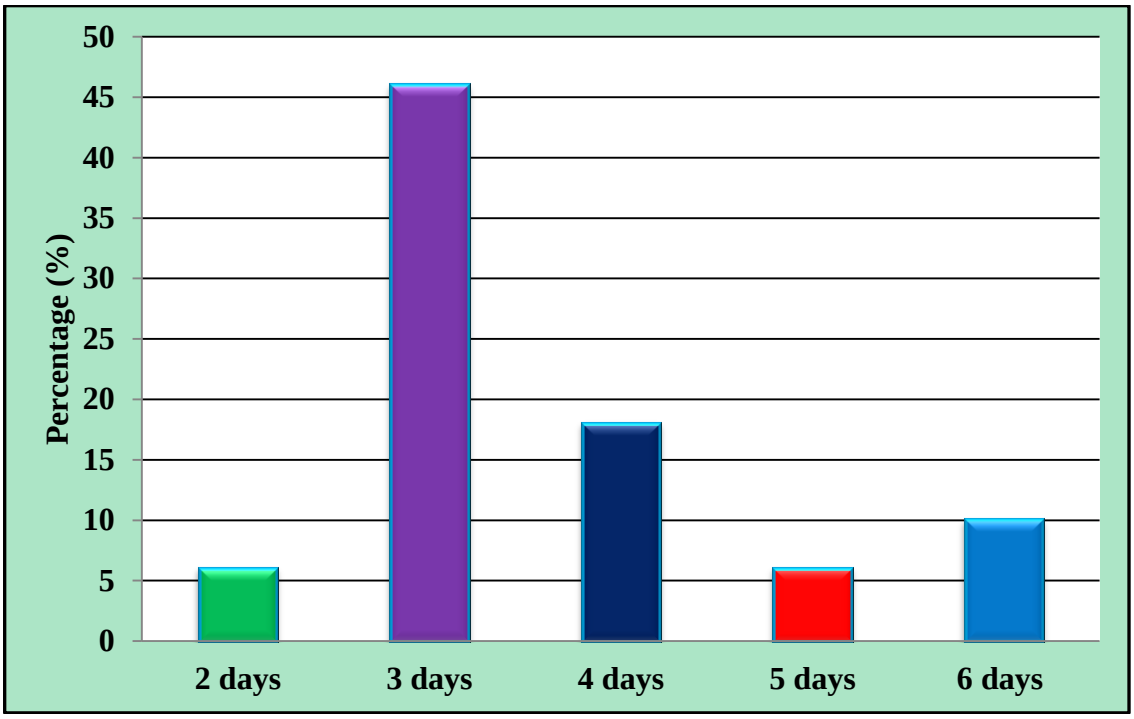
Graph-9: Distribution of patients according to WBC count and distribution



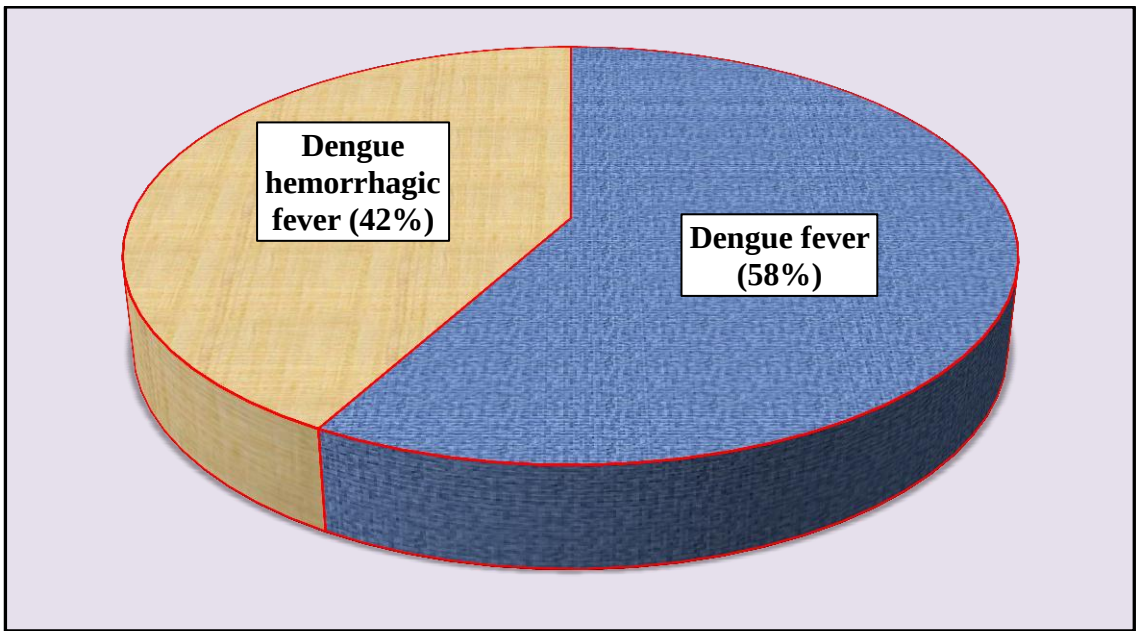
Graph-10: Distribution of percentage of patients according to units of platelet transfusion



Graph-11: Distribution of percentage of patients according to the number of days to return to normal platelet count



Graph-12: Distribution of percentage of patients according to diagnosis



Graph-13: Distribution of percentage of patients according to recovery from dengue feve

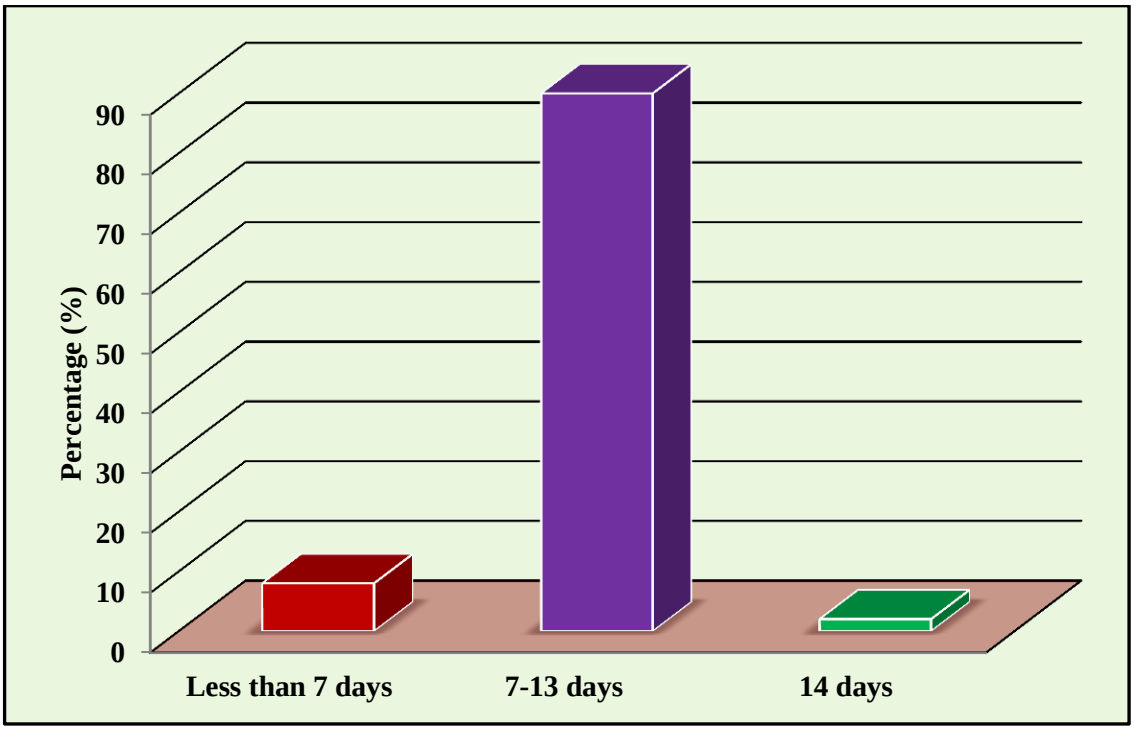
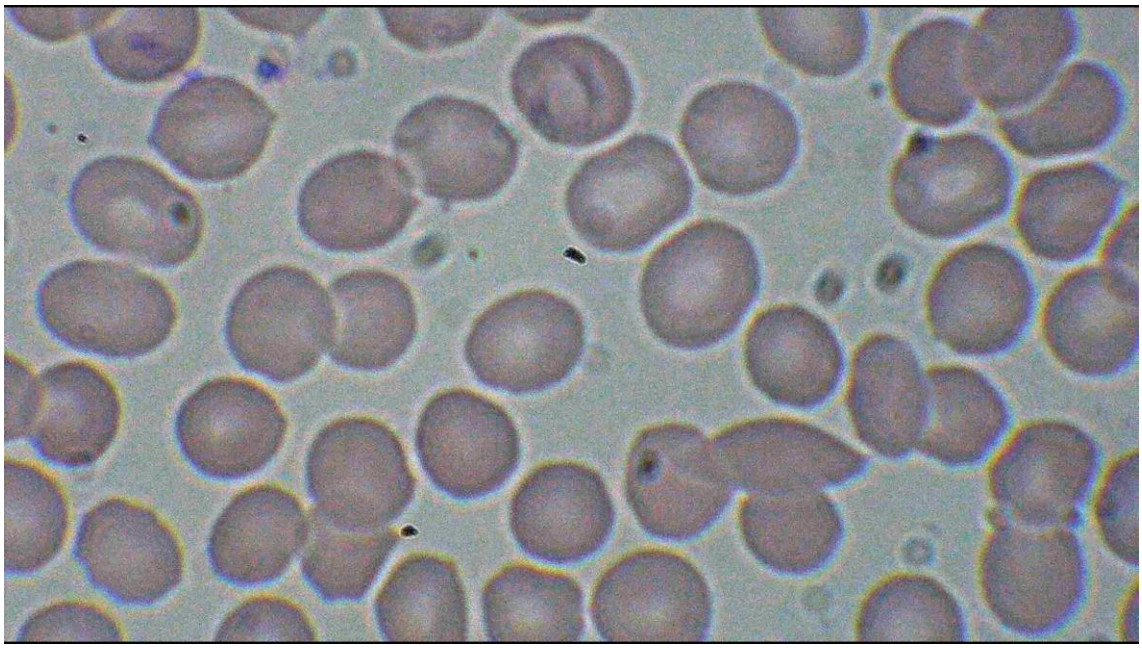


Fig 1:Peripheral blood smear showing severe thrombocytopenia and leukopenia(100 X)



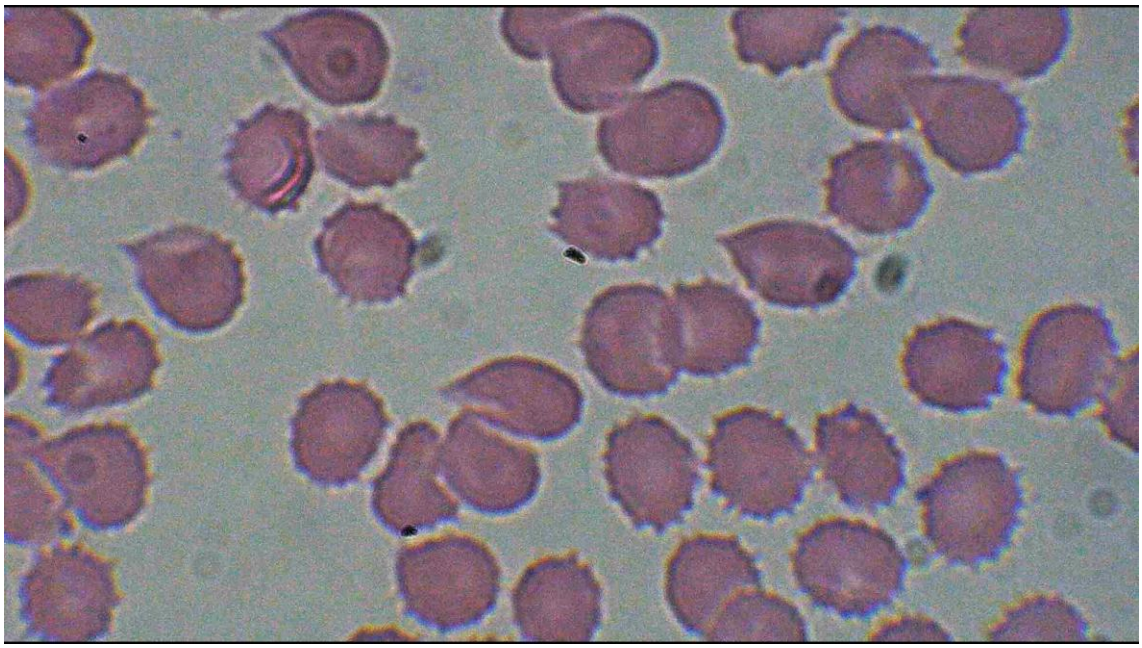
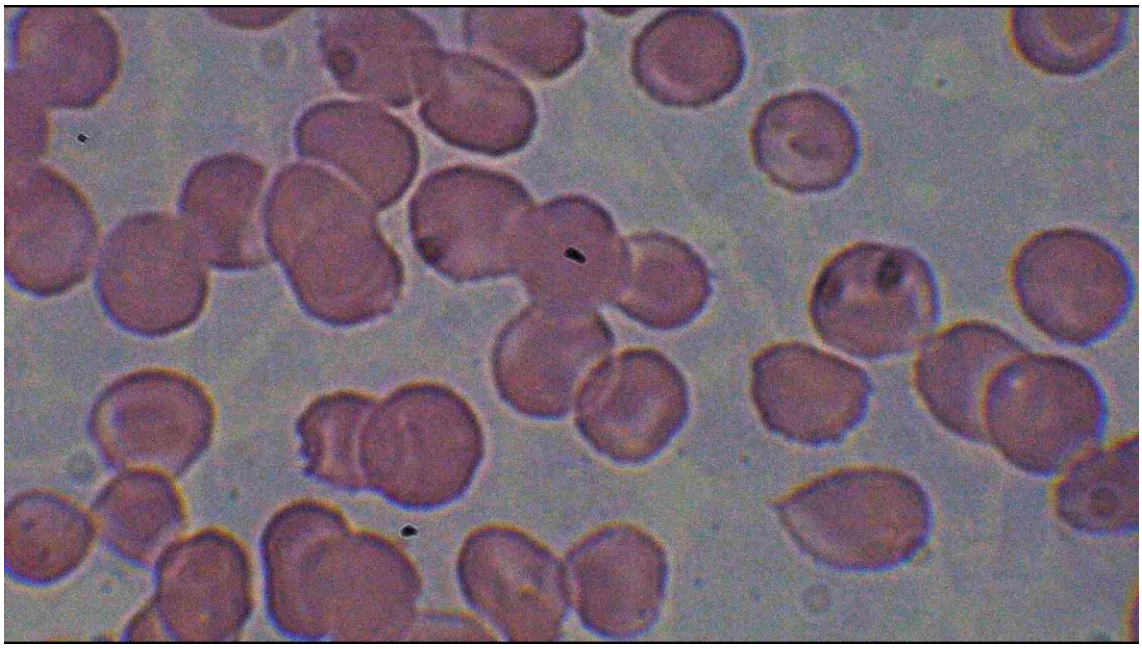


Fig 2:Peripheral blood smear showing severe thrombocytopenia with tear drop cells,crenated RBC's (100 x)

Fig 3:Another case of thrombocytopenia with severe thrombocytopenia and leukopenia(100x)



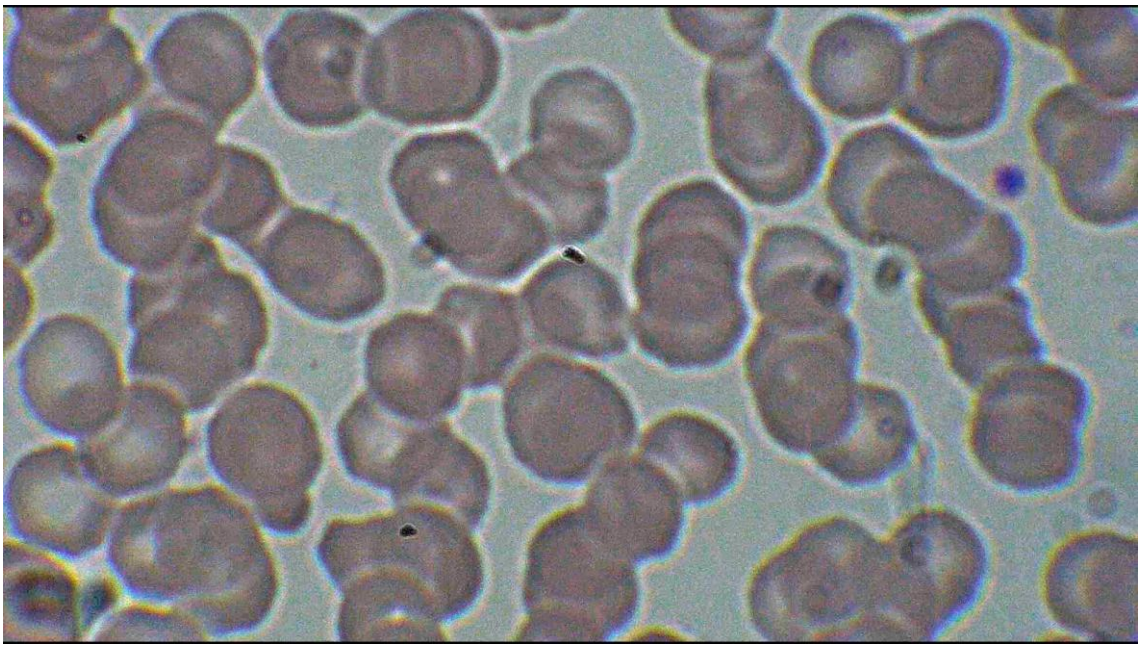
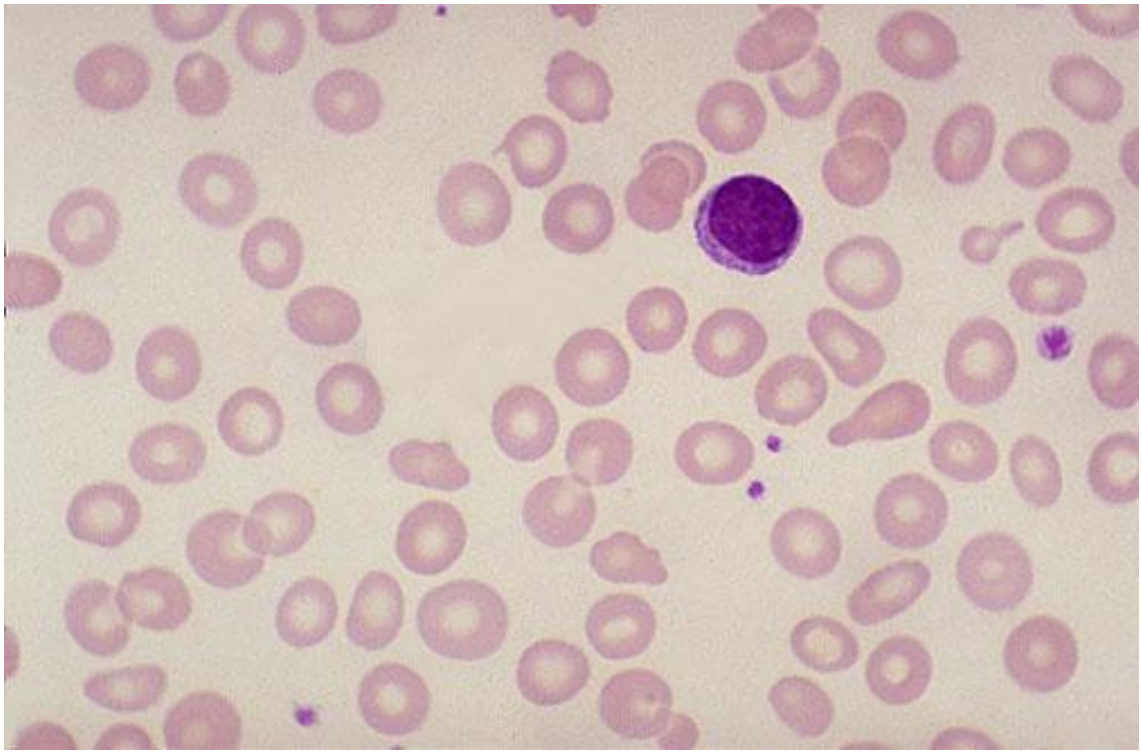


Fig 4: Peripheral blood picture showing closely packed RBC's due to haemoconcentration (100x)

Fig 5:Peripheral blood smear showing microcytic hypochromic RBC's with moderate to severe thrombocytopenia(100x)



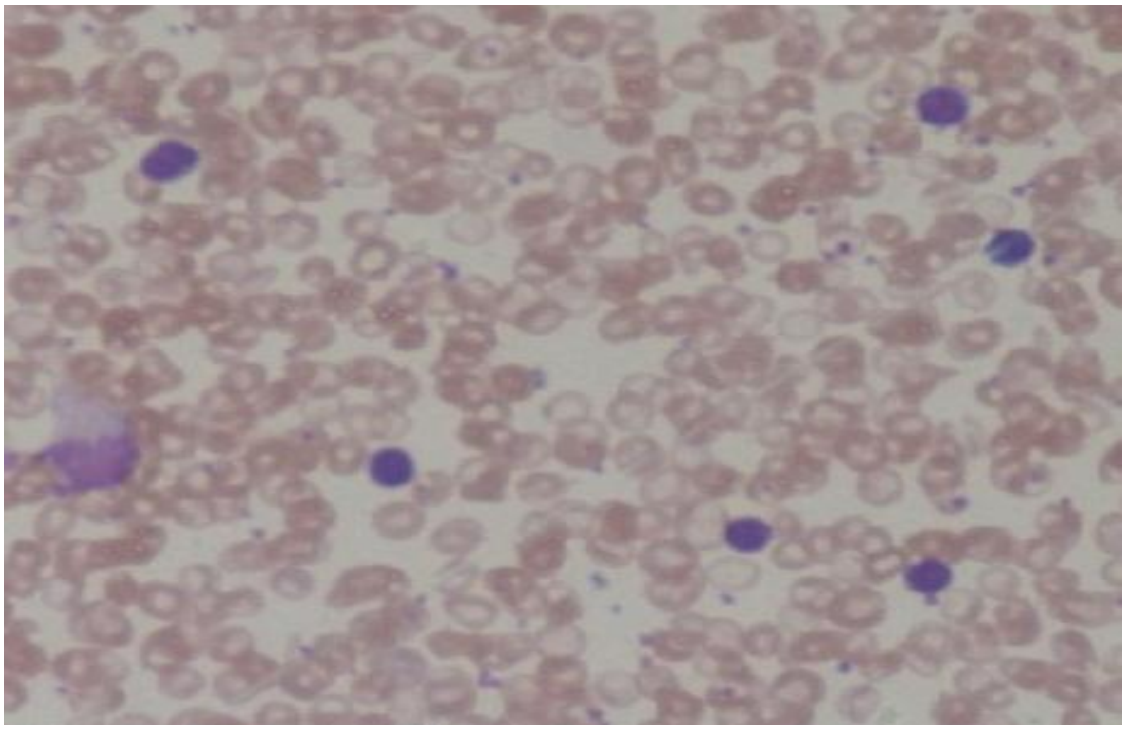
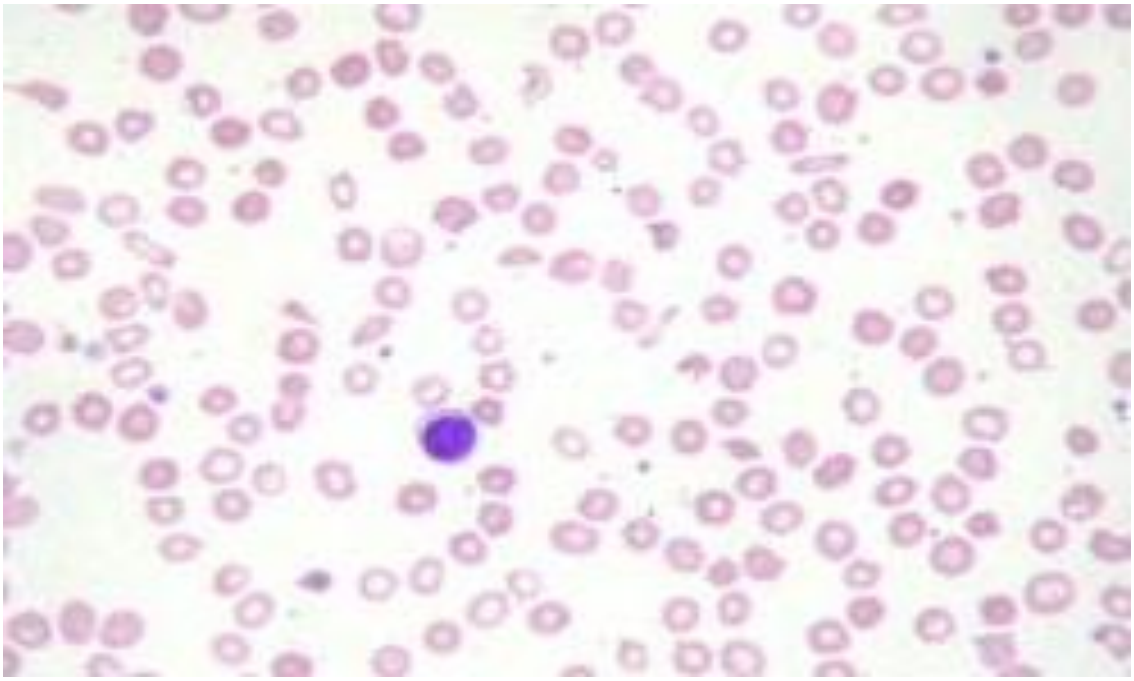


Fig 6:Peripheral blood picture showing lymphocytosis and microcytic hypochromic RBC's(100x)

Fig 7:Microcytic hypochromic blood picture with mild thrombocytopenia(100x)



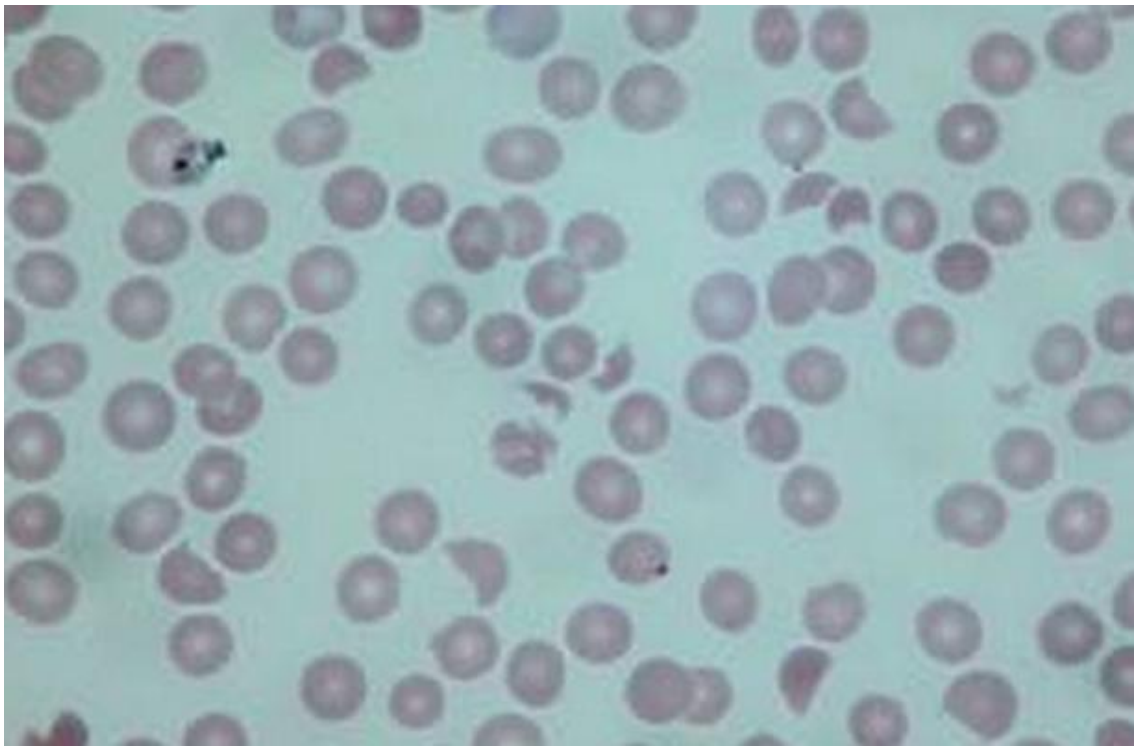


Fig 8: Marked thrombocytopenia with leucopenia(100x)

Dengue fever remains the most important of the arboviral infections with significant socioeconomic and healthcare implications. The clinical spectrum ranges from non-specific viral syndrome to classical dengue fever to life threatening denguehaemorrhagic fever and dengue shock syndrome. Early diagnosis and aggressive management especially for DHF and DSS remains the cornerstone strategy for successful outcomes.

A total of 50 patients admitted to our hospital with symptoms of dengue fever and positiveNS1/ IgM ELISA were studied

Age:

The average age of patients in our study group was 36.84 years. In the study by Nadeem MA et al.[74]the mean age was 36 years, where as the mean age was 34.7 years according to the study by Tiwari KN et al.[57]

Gender:

Out of the 50 cases included in our study 29(58%) were males and 21(42%) were females.in comparison the study done by Nadeem MA et.al [74] the gender ratio was 390(70%) in males and 166(30%) in females of the 556 cases.The sex distribution study by Raju BJ et.al[58] were as follows,out of the 200 patients included 121 (60.5%) were males 79(39.5%) were females.Our studies had a male preponderance in comparison to the other studies.

CLINICAL FEATURES:

Fever:

All patients in our study presented with fever on admission.Similarly studies done by Turbadkar D et .al ,[68]Mandal SK et. al ,[70]Karyawasam S et al [73,]Nadeem MA et .al,[74]Tiwari KN et .al[57] and Raju BJ et .al [58] had all their patients(100%) with fever followed by Fatima S et .al [71](99%),Lt Col. Banerjee M et.al [63] (85 %) and Khan AH et.al [69] (80%).Our study correlated well with most of the studies.

Headache:

Headache was universally present among all our patients (100%).In other studies the incidence of headache was as follows ,Raju BJ et. al [58] (98%), Lt .Col. Banerjee M et .al [63](63%), Mandal KS et.al [70] (62.16%), Khan AH et al[69](50%),Turbadkar D et .al[68](13.9%).Our study differed from most of the other studies as headache was not a prominent symptom in those studies.

Retroorbital pain:

This symptom is considered a hallmark of Dengue fever.The number of patients in our study manifesting this were 7(14%) out of the 50 cases. Raju BJ et.al[58] in their study of 200 dengue cases reported a 26.5% of incidence of retroorbital pain.

Myalgia:

Our study revealed that 43(86%) out of 50 patients had myalgia.Studies done by Raju BJ et.al [58] had 85.5% of patients having myalgia,Lt.Col.Banerjee M et .al [63](81%), KariyawasamS etal[73](70%), Turbadkar D et al[68](25%),.Tiwari

KN et al[57](24%) and Khan AH et al[69](10%) of the patients presented with myalgia.

Arthralgia:

5(10%) of the patients had arthralgia. Raju BJ et.al [58] reported a incidence of 16% among their study group.

RESPIRATORY COMPLAINTS:

URTI/LRTI:

We encountered 6(12%) of our patients with URTI; and 8(16%) had lower respiratory tract infection. Khan AH et al [69] reported 35% manifesting as URTI in their study.

GASTROINTESTINAL COMPLAINTS:

Abdominal pain:

We had 14(24%) of patients with abdominal pain. Raju BJ et.al[58] has had an incidence of 55% and Tiwari KN et .al[57] with 34% of the patients presenting with abdominal pain

Nausea and vomiting:

20(40%) of our patients experienced the above mentioned symptoms. Raju BJ et al[58] reported a incidence of (84.5%), Khan AH et.al[69](60%), Tiwari KN et .al[57](36%).

Diarrhoea:

Loose stools were seen in 7(14%) of the patients.None of the other studies reported diarrhea in their study group.

Hepatomegaly:

Hepatomegaly was observed in 26(52%) of our patients. Raju BJ et .al[58] (30%) and Lt.Col.Banerjee.M et.al [63]reported an incidence of 15% among their patients.

Splenomegaly:

Enlarged spleen was palpable in 24(48%) our patients.Lt.Col. Banerjee M et.al [63] reported splenomegaly in 7% of the patients.

SIGNS OF CAPILLARY LEAK:

Evidence of capillary leak is the hallmark of DHF and DSS .The three common signs are a rising hematocrit,ascites and pleural effusion.

Pleural effusion:

Pleural effusion was assessed by either chest radiograph or by ultrasound examination.8(16%) of our patients had pleural effusion. Fatima S et.al[71] reported a incidence of 19%(25 out of 131 cases)

Ascites:

Ascites was assessed by ultrasonographic examination.A total of 13(26%) patients had ascites in our study group. Raju BJ et.al [58] reported a incidence of 21.5% out of 121 dengue positive patients Fatima S et.al[71] reported an incidence of 11.5% (15 out of 131 cases) in their study.

Rising haematocrit:

Significant or rising haematocrit was seen in 8(16%) of our patients. Fatima S et.al[71] reported an incidence of 73% in their study. Nadeem MA et.al[74] in their analysis of dengue patients at two major public sector tertiary care

hospitals in Lahore,Pakistan, reported a rising haematocrit in 21% of patients out of 390 patients.

SEROLOGY:

We confirmed a diagnosis of dengue fever with NS1 ELISA and IgM ELISA tests, but included patients who were IgM positive.Out of the 50 patients 29(58%) were IgM only positive and the rest 21(42%) were both NS1 and IgM positive. Bandyopadhyay B et.al[55] in their study at Calcutta School of Tropical Medicine,screened patients suggestive of DF with NS1 and IgM ELISA test and included 64 patients out of which 38 were NS1 positive.Out of the 38 cases,24 were found to be IgM positive and the rest 14 cases were IgM ELISA negative.This was attributed to the possible secondary dengue infection where the IgM titres sometimes become undetectable. Fatima S et.al [71] screened patients with the symptoms of dengue fever with IgM ,IgG ELISA and NS1 ELISA tests and included 131 cases who wre serologically positive for DF.Out of the 131 patients 35(27%) were positive for IgM only,63(48%) positive for both IgM and 65(50%) were NS1 positive. Turbadkar D et.al[65],screened a total of 3,677 patients with symptoms consistent with DF with IgM and IgG ELISA tests and found a total of 503 serologically positive cases .Out of these 288(57.25%) were positive for IgM ELISA alone ,followed by 108 cases (21.4%) for IgG ELISA and 107 (21.4%) for both IgM and IgG ELISA.The authors have excluded patients with isolated IgG positivity as it may be due to a remote dengue infection.

HAEMATOLOGICAL PARAMETERS:

Anemia:

Our study revealed an incidence of 8%(4 patients) whereas the study conducted by Lt.Col.Banerjee M.et.al [63] revealed an incidence of 11% in 50 patients studied.

Leukopenia:

Leukopenia was seen in 22 (44%) of our patients,whereas in the study by Lin SF et.al [67],the incidence of leucopenia was 76% in patients with dengue fever.According to a study by Lt.Col.Banerjee M et.al[63],none of the 50 cases of DF manifested leucopenia.

Thrombocytopenia:

Thrombocytopenia was observed in 48 (96%) in our study,Based on the grading 12(25%) of cases showed grade 1 thrombocytopenia,5(10.42%) showed grade 2,22(45.83%) Grade 3 and 22(45.83%) Grade 4.Turbadkar D.et.al [68] in their study of 212 patients reported an incidence of 76.74% of thrombocytopenia.Sarwat Fatima et.al [70] reported thrombocytopenia to the tune of 89% in their study involving a total of 131 seropositive patients. Kariyawasam S et.al [73] reported a100% incidence of thrombocytopenia in their study involving 15 pregnant patients who were seropositive for DF. Nadeem MA et.al [74] in their study reported an incidence of 93% of thrombocytopenia in their study involving 556 seropositive patients during the 2011 dengue fever epidemic in Lahore,Pakistan. Khan MU et.al [80] in their study quoted a 67.2% thrombocytopenia among 210 seropositive dengue positive patients. Khan DM et.al [83] reported an incidence of 71% thrombocytopenia.

BIOCHEMICAL PARAMETERS:

Liver function tests:

Hyperbilirubinemia (S.Bilirubin) was seen in 12(24%) of the patients in our study.None of the studies quoted have described jaundice in these patients.

SGOT(AST) AND SGPT(ALT):

Transaminitis was encountered in many of our patients.SGOT levels were elevated in 39(78%) and elevated SGPT levels were seen in 38(76%) of our patients. Mandal SK et .al[70] in their study reported transaminitis of 83.83%. Kariyawasam S et.al[73] reported a 100% incidence of elevated liver enzymes(SGOT,SGPT) in their study involving 15 seropositive pregnant women.In our study the mean SGOT(AST) levels were 141.60 IU/L.SGPT(ALT) levels were 118.54 IU/L. Prakash O et.al [76] in their exclusive study of hepatitis in dengue fever in a tertiary care hospital in Karachi,Pakistan,involving 699 seropositive cases reported a mean SGOT(AST) of 174 IU/L and SGPT(ALT) of 88.50 IU/L.

COAGULATION PROFILE:

Prothrombin Time(PT-INR) and activated partial Thromboplastin time(aPTT) :

The normal prothrombin time INR value is 0.8 to 1.2 .7(14%) of our patients had prolonged prothrombin time.

Activated partial thromboplastin time(aPTT) was prolonged in 10(20%) of our patients.None of the studies reported significant abnormalities in coagulation profile.

RENAL FUNCTION TESTS:

Serum urea and creatinine:

We have encountered mild renal failure in few of our patients who all had complete recovery of renal function during discharge or followup.Blood urea was elevated in 10(20%) and serum creatinine was elevated in 9(18%) of the patients.There is no clear data regarding this in those studies quoted.

PLATELET TRANSFUSION:

Indication:

A total of 43 patients (86%) received platelet transfusion in our study group.The sole indication being moderate to severe thrombocytopenia.

Tiwari KN et.al [57] in their retrospective study thrombocytopenia was the common indication for platelet transfusion in 4 tertiary care hospitals in Delhi.A total of 230 seropositive cases were included and out of which 130 cases(56.5%) received platelet transfusion.The common indication was thrombocytopenia rather than bleeding manifestation. Kulkarni.N.[84] in her study 118(51%) out of 232 patients received single unit platelet transfusion with platelet counts between 20,000 and 1.00.000cells/cu.mm and 64(27.5%) with platelet of <20,000 cells/cu.mm received multiple platelet transfusions.It was concluded that 51% of platelet transfusions were inappropriate and more effective when it is given with a platelet count of <20,000cells/cu.mm.

Units of platelets transfused:

In our study 54%(27) received 1-2 units,10%(5) received 3-4 units,16%(8) of cases were given 5-6 units,4%(2) were given 7-8 units and 2%(1) received >9 units of platelet transfusion.The mean units of platelets transfused is 2.42 units .The average number of days of hospital stay for those who received platelet transfusion 5 days and who had not receive platelet transfusion was 3.7 days. Tiwari KN et .al [57] in his study of 230 seropositive dengue patients 130 received platelet transfusion,the minimal units of platelets transfused was one and maximum of 16 units was transfused.The average number of days in the hospital for those who received platelet transfusion was 4.9 days as compared to a longer duration of hospital stay(5.3days) for those who did not receive platelet transfusion. Kulkarni.N.[84] in her study 118(51%) out of 232 patients received single unit platelet transfusion with platelet counts between 20,000 and 1.00.000cells/cu.mm and 64(27.5%) with platelet of <20,000cells/cu.mm received multiple platelet transfusions.It was concluded that 51% of platelet transfusions were inappropriate and more effective when it is given with a platelet count of 20,000.

PROFILE:

The number of patients who had dengue fever were 29(58%) out the total 50 patients and DHF was 21(42%).None of the patients had DSS in our study group. Fatima S et.al[71] in their study of 131 seropositive dengue case had DF(60%) ,DHF(35.5%) and DSS(4.5%). Arif M et .al [74] in the study of 556 seropositive dengue cases 439(78%) had DF,95(17%) had DHF and 26(4%) has DSS. Khan DM et.al [83] in their study of 107 seropositive cases in a teaching

hospital in rural Melmaruvathur in Tamilnadu,out of these 58(54.2%) had DF,38(35.5%) had DHF and 11(10.2%) had DSS.

Hati AK et.al [56] in their study in West Bengal had 90% of patients having DF and 10% had DHF. Tiwari KN et al[57] in their study based in Delhi,included 230 seropositive cases.Out of these 163(70.8%) had DF and 29.2% had DHF.

Dengue has evolved into a truly global arboviral illness with ever increasing multiple epidemics and a rising incidence of severity in the form of Dengue Haemorrhagic Fever throughout the tropics and subtropics with an impending risk of invading the temperate regions in this era of climate change and global warming.

Our study focused on the haematological profile of dengue fever in correlation with the clinical course. It was concluded in this study that fever with headache in the presence of thrombocytopenia ,leukopenia and reversal of neutrophil lymphocyte ratio should raise a strong suspicion of Dengue fever for which screening and confirmation must be done.

Signs of capillary leak like rising haematocrit, ascites and pleural effusion should alert the treating physicians for prompt and aggressive management for the high risk of Dengue Haemorrhagic Fever.

Thrombocytopenia and a rising haematocrit (PCV) by 20% from baseline were found to be important prognostic indicators during hospital stay and followup.

Platelet transfusion, another focus of this study, was initiated in patients with moderate to severe thrombocytopenia. The patients had a smooth and rapid recovery with no adverse outcomes related to the transfusion.

All patients with abnormalities in the clinical, haematological and biochemical parameters in our study group made a complete recovery either during the hospital stay or within the follow up period.

In conclusion early suspicion, screening, diagnosis and prompt management of Dengue fever will effectively lower any morbidity or mortality related to this dreaded illness.

- A total of 50 patients who were admitted with fever and IgM ELISA with or without NS1 antigen positivity were studied.
- Out of the fifty patients 29(58%) were diagnosed to have dengue fever(DF),and the rest 21(42%) had Dengue Haemorrhagic Fever(DHF)
- The age distribution was between14-70 years with more than 50% falling between 21-40 years age group.
- The male to female ratio is 1.3:1 in this study.
- The common clinical manifestations were fever(100%) and headache(100%),followed by myalgia(86%),hepatomegaly(52%) and splenomegaly(48%).
- The common signs of capillary leak were ascites (26%),rising haematocrit and pleural effusion each (16%)
- Bleeding manifestations commonly encountered were minor manifestations like petichiae(16%),only (2%) had major bleeding manifestation like GI bleed.(82%) showed no haemorrhagic manifestations.
- All the patients who were serologically positive for dengue IgM ELISA were chosen out of which 21(42%) tested positive for NS1 antigen also.
- The common haematological abnormalities encountered were thrombocytopenia in 48(96%) patients, leucopenia in 22(44%) of patients, followed by anemia in 14(28%) of patients.
- Thrombocytopenia observed: Grade 1 was 12(25%), Grade 2 was 5(10.42%)Grade 3 was 22(45.83%) and Grade 4 9(18.75%).
- Leukopenia with reversal of neutrophil lymphocyte ratio was seen in 6(12%) of the patients.

- Abnormalities in liver function parameters(hepatitis) was the most common biochemical abnormality detected. Hyperbilirubinemia was detected in 12(24%) of the patients, raised AST(SGOT) in 39 (78%) and raised ALT (SGPT) in 38(76%) of patients.
- Coagulation profile derangements were as follows: Prolonged prothrombin time (PT- INR) was detected in 7(14%) of the patients and elevated aPTT was seen in 10(20%) of the patients.
- Renal function abnormalities including raised blood urea was seen in 10(20%) of the patients and elevated creatinine levels were detected in 9(18%) of the patients.
- The number of patients who received platelet transfusion were 43(86%).
- The common indication for platelet transfusion was moderate to severe thrombocytopenia followed by bleeding tendencies.
- Most of the patients, 26(52%) out of fifty were initiated platelet transfusion on the second day following admission. The number of platelets transfused ranged from 1-10 units, but most of them 27(54%) received only 1-2 units of platelets.
- Normalization of platelet count for those who received platelet transfusion occurred between 2-6 days after initiation of transfusion. The commonest being on the third day,23(53.49%) out of 43 patients.
- None of the patients who received platelet transfusion developed any form of transfusion reaction in our study
- The average duration of hospital stay was 8.7 days.
- None of the patients developed Dengue Shock Syndrome(DSS) and all the patients made a complete recovery.

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Institutional Human Ethics Committee

Ref. No. SMIMS/IHEC/2013/A/07 **Date: 1st July 2013**

Certificate

This is to certify that the Research Protocol Ref. No. **SMIMS/IHEC/2013/A/07,** entitled "Dengue: A Clinicohaematological Profile and Role of Platelet Transfusion in it's Management" submitted by Dr. Evelyn Angel S, Postgraduate of Department of Pathology, SMIMS has been approved by the Institutional Human Ethics Committee at its meeting held on 30th of May 2013.

[This Institutional Human Ethics Committee is organized and operates according to the requirements of ICH-GCP/GLP guidelines and requirements of the Amended Schedule-Y of Drugs and Cosmetics Act, 1940 and Rules 1945 of Government of India.]

SMIMS INSTITUTE OF MEDICAL SCIENCES IHEC Date...5/13 KULASEKHARAM

Dr. Rema Menon. N
Member Secretary
Institutional Human Ethics Committee
Professor of Pharmacology and HOD
SMIMS, Kulasekharam (K.K District)
Tamil Nadu -629161

ANNEXURE-I

PARTICIPANTS INFORMED CONSENT FORM

The details of the study have been explained to me in writing and the details have been fully explained to me. I an aware that the results of the study may not be directly beneficial to me but will help in the advancement of medical sciences. I confirm that I have read and understood the information sheet for the above study and had the opportunity to ask questions. I understand that my participation in the study is voluntary and I am free to withdraw at any time without giving any reason, without medical care that will be normally provided by this hospital being affected. I agree not to restrict use of any data or results that arise from this study provided such a use is only for scientific purpose(s).I have been given an information sheet giving the details of the study .I fully consent to participate in the study titled **"Dengue: A Clinicohaematological profile and role of platelet transfusion in its management."**

Serial Number/ Reference No :

Name of the Participant :

Address of the participant :

Contact number of the participant :

Signature/ Thumb impression of the participant/ Legal guardian
(if below 18 years)

Witness 1 :

Witness 2 :

Date :

Place :

ANNEXURE-I

PROFORMA

Dengue: A Clinicohematological Profile and Role of Platelet Transfusion in Its Management

Name of the Patient: OP No.:

Age : IP No.:

Sex : DOA :

Occupation : DOD :

Address :

Chief Complaints :

History of Presenting Illness:

(A) Constitutional Symptoms

1. Fever : Present ☐ ; Absent ☐
2. Duration of Fever : ☐ days
3. Musculoskeletal Symptoms

   Headache : Present ☐ ; Absent ☐

   Retroorbital Pain : Present ☐ ; Absent ☐

   Myalgia : Present ☐ ; Absent ☐

   Arthralgia : Present ☐ ; Absent ☐

4. Symptoms of Spontaneous Bleeding

   Skin petichaes : Present ☐ ; Absent ☐

   Ecchymosis : Present ☐ ; Absent ☐

   Epistaxis : Present ☐ ; Absent ☐

   Oral bleed : Present ☐ ; Absent ☐

   Hemoptysis : Present ☐ ; Absent ☐

   Hemetemesis : Present ☐ ; Absent ☐

   Melena : Present ☐ ; Absent ☐

   Hematuria : Present ☐ ; Absent ☐

5. Respiratory Symptoms

URTI	:	Present ☐ ; Absent ☐
LRTI	:	Present ☐ ; Absent ☐
Dyspnoea	:	Present ☐ ; Absent ☐

6. Gastrointestinal Symptoms

Abdominal pain	:	Present ☐ ; Absent ☐
Diarrhoea	:	Present ☐ ; Absent ☐
Vomiting	:	Present ☐ ; Absent ☐

7. Other Manifestation

Family History:

Personal History :

Diet : Sleep :

Appetite : Habits :

Bowl and Bladder:

Treatment History :

History of Underlying Illness:

General Physical Examination

Pallor	:	Icterus:		Cyanosis	:
Clubbing	:	Lymphadenopathy:		Oedema	:

Maculopapular Rash:

Petichaes:

Vitals

Pulse Rate:	Respiratory Rate:
Blood Pressure:	Temperature:

Systemic Examination

Cardiovascular System :

Respiratory System :

Abdomen :

Central Nervous system :

INVESTIGATIONS

Hematological Parameters

1. NS1 Antigen : Positive ☐ Negative ☐
2. IgM Antibody : Positive ☐ Negative ☐
3. Hemoglobin : gm%
4. Packed Cell volume
   - Hematocrit : Significant ☐ Insignificant ☐
   - Rising titre : Present ☐ Absent ☐
5. Total WBC count : cumm/L
6. Differential WBC count
   - Polymorphs :
   - Lymphocytes :
   - Eosinophils :
   - Basophils :
   - Monocytes :
   - Other forms :
7. Platelet count : cumm/L
8. Peripheral Smear :

Biochemical Parameters

1. AST	:	S.Bilirubin	:
2. ALT	:	BUN	:
3. aPTT	:	S.Creatinine	:
4. PT	:	Blood glucose	:

Other Parameters

1. Chest Radiograph**:**

2. Ultrasonography of Abdomen and Pelvis:

Vital Statistics

A.	Signs of Capillary Leak (Raising Hematocrit, Ascites, Pleural Effusion)	:	Present	☐	Absent	☐
B.	Hemorrhagic Manifestation	:	Present	☐	Minor	☐
			Major	☐	Absent	☐
C.	Significant Shock	:	Present	☐	Absent	☐

GRADING OF DENGUE FEVER

1. Dengue Fever (DF) ☐
2. Dengue Hemorrhagic Fever (DHF) ☐
3. Dengue Shock Syndrome (DSS) ☐

PLATELET TRANSFUSION DETAILS(IF GIVEN)

I.	Platelet Transfusion	: Done	☐	Not Done	☐
II.	Indication for Platelet Transfusion	:			
III.	Level of Thrombocytopenia at Which Platelet Transfusion was Initiated	:			

IV. Day at Which Platelet Transfusion was Initiated ☐

V Patients Response to Platelet Transfusion

No of Days	0	1	2	3	4	5
Units of platelet transfused						
Patient'splatelet Count						

VI. Adverse Events Related to platelet Transfusion :

FINAL OUTCOME

Recovered ☐

Expired ☐

Date:

Place:

Signature of Researcher

ANNEXURE-II (MASTER CHART)

Sl. No	Age	Sex	Duration of fever	Day of Discharge	CLINICAL FEATURES												Haemorrhagic manifestations			Signs of Capillary Leak			
					Musculoskeletal				Respiratory			Gastrointestinal					Present		Absent	Pleural effusion	Ascites	Rise of heamato crit	Shock
					Headache	Retroorbital pain	Myalgia	Arthralgia	URTI	LRTI	Dyspnoea	Abdominal pain	Vomiting	Diarrhoea	Hepatomegaly	Splenomegaly	Major	Minor					
1.	29	F	6 days	7	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2.	47	M	8 days	9	+	-	+	-	-	+	-	-	+	-	+	+	-	-	-	-	-	-	-
3.	60	M	5 days	7	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4.	41	M	5 days	6	+	-	+	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-
5.	44	M	10 days	12	+	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
6.	14	M	5 days	8	+	-	+	-	-	-	-	+	-	+	+	+	-	-	-	-	-	-	-
7.	27	F	6 days	6	+	-	+	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-
8.	32	M	7 days	8	+	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
9.	27	F	5 days	6	+	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-

ANNEXURE-II (MASTER CHART)

Sl. No	Age	Sex	Duration of fever	Day of Discharge	CLINICAL FEATURES												Haemorrhagic manifestations			Signs of Capillary Leak			
					Musculoskeletal				Respiratory			Gastrointestinal					Present		Absent	Pleural effusion	Ascites	Rise of heamato crit	Shock
					Headache	Retroorbital pain	Myalgia	Arthralgia	URTI	LRTI	Dyspnoea	Abdominal pain	Vomiting	Diarrhoea	Hepatomegaly	Splenomegaly	Major	Minor					
10.	38	F	6 days	7	+	-	+	-	-	-	-	-	+	-	+	+	-	+	-	-	+	+	-
11.	15	F	7 days	8	+	-	+	-	-	-	-	-	+	-	+	+	-	+	-	+	-	+	-
12.	22	F	6 days	7	+	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-
13.	39	M	5 days	7	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-
14.	24	M	6 days	7	+	-	-	+	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-
15.	68	M	5 days	6	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
16.	15	F	5 days	7	+	-	+	-	-	-	-	-	-	-	+	+	-	-	-	-	+	+	-
17.	33	M	6 days	7	+	-	+	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-
18.	39	M	10 days	11	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-

ANNEXURE-II (MASTER CHART)

Sl. No	Age	Sex	Duration of fever	Day of Discharge	CLINICAL FEATURES												Haemorrhagic manifestations			Signs of Capillary Leak			
					Musculoskeletal				Respiratory			Gastrointestinal					Present		Absent	Pleural effusion	Ascites	Rise of heamato crit	Shock
					Headache	Retroorbital pain	Myalgia	Arthralgia	URTI	LRTI	Dyspnoea	Abdominal pain	Vomiting	Diarrhoea	Hepatomegaly	Splenomegaly	Major	Minor					
19.	70	M	6 days	7	+	-	+	-	+	+	-	-	-	-	+	+	-	+	-	-	+	-	-
20.	16	M	7 days	8	+	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-
21.	31	M	6 days	7	+	-	+	-	+	+	-	-	-	+	-	+	-	-	-	-	+	+	-
22.	27	M	5 days	7	+	-	-	+	-	+	-	+	+	-	+	+	-	+	-	-	+	-	-
23.	62	F	5 days	7	+	+	-	+	-	-	-	+	+	-	+	+	-	+	-	+	+	-	-
24.	18	M	7 days	8	+	+	+	-	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-
25.	55	F	7 days	8	+	-	+	-	-	-	-	+	+	-	-	-	-	-	-	+	+	-	-
26.	30	F	5 days	7	+	-	+	-	-	-	-	-	+	-	+	+	+	-	-	-	-	-	-
27.	44	M	6 days	7	+	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	+	+	-

ANNEXURE-II (MASTER CHART)

Sl. No	Age	Sex	Duration of fever	Day of Discharge	CLINICAL FEATURES												Haemorrhagic manifestations			Signs of Capillary Leak			
					Musculoskeletal				Respiratory			Gastrointestinal					Present		Absent	Pleural effusion	Ascites	Rise of heamato crit	Shock
					Headache	Retroorbital pain	Myalgia	Arthralgia	URTI	LRTI	Dyspnoea	Abdominal pain	Vomiting	Diarrhoea	Hepatomegaly	Splenomegaly	Major	Minor					
28.	65	M	7 days	8	+	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
29.	36	F	6 days	9	+	-	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
30.	17	M	7 days	9	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31.	27	M	6 days	8	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32.	19	M	5 days	7	+	-	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-
33.	60	F	7 days	8	+	-	+	-	-	-	+	+	+		+	+	-	-	-	-	-	-	-
34.	29	M	8 days	10	+	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
35.	38	F	6 days	8	+	-	+	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-
36.	47	F	10 days	12	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-

ANNEXURE-II (MASTER CHART)

Sl. No	Age	Sex	Duration of fever	Day of Discharge	CLINICAL FEATURES												Haemorrhagic manifestations			Signs of Capillary Leak			
					Musculoskeletal				Respiratory			Gastrointestinal					Present		Absent	Pleural effusion	Ascites	Rise of heamato crit	Shock
					Headache	Retroorbital pain	Myalgia	Arthralgia	URTI	LRTI	Dyspnoea	Abdominal pain	Vomiting	Diarrhoea	Hepatomegaly	Splenomegaly	Major	Minor					
37.	19	M	6 days	7	+	-	+	-	-	-	+		+	+	-	+	-	-	-	-	-	-	-
38.	29	F	7 days	9	+	-	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
39.	21	M	5 days	7	+	-	+	-	-	-	-	-	+	+	-	+	-	-	-	-	+	+	-
40.	34	F	6 days	7	+	-	+	-	-	-	+	+	+		-	-	-	-	-	-	-	-	-
41.	40	M	10 days	12	+	-	+	-	+	+	-	-	+	+	-	+	-	+	-	+	+	-	-
42.	68	F	6 days	7	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
43.	23	M	6 days	8	+	+	+	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-
44.	32	F	7 days	9	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	+	+	-	-
45.	30	M	7 days	8	+	-	+	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-

ANNEXURE-II (MASTER CHART)

Sl. No	Age	Sex	Duration of fever	Day of Discharge	CLINICAL FEATURES												Haemorrhagic manifestations			Signs of Capillary Leak			
					Musculoskeletal				Respiratory			Gastrointestinal					Present		Absent	Pleural effusion	Ascites	Rise of heamato crit	Shock
					Headache	Retroorbital pain	Myalgia	Arthralgia	URTI	LRTI	Dyspnoea	Abdominal pain	Vomiting	Diarrhoea	Hepatomegaly	Splenomegaly	Major	Minor					
46.	45	F	5 days	7	+	-	+	-	-	-	-	-	-	+	-	+	-	-	-	+	-	+	
47.	76	F	7 days	14	+	+	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	+	+
48.	39	M	6 days	8	+	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
49.	36	F	8 days	9	+	+	+	-	-	-	-	-	+	+	-	+	-	+	-	-	-	-	-
50.	45	M	9 days	10	+	+	+	-	-	-	+	-	-	-	-	-	-	+	-	-	+	+	-

ANNEXURE-II (MASTER CHART)

Haematological Parameters												Biochemical parameters																				
						Differential Count																Done										
Sl No.	NS1Antigen	IgM	Hb%	PCV	WBC	Poly	Lymph	Eosino	Monocytes	Baso	Platelet Count	P.Smear	AST (SGOT)	ALT (SGPT)	S.Bilirubin	S.Urea	S.Creatinine	APTT	B.Glucose	PT INR	Not Done	Indication	Day of Initiation	Thrombocytopenia	No.of units	Day of Normal Platelet	Adverse effects	Profile	Final Outcome
1.	+	+	10.9	32.1	9700	57.9	32.3	2.2	7	0.5	232000	NNA,WBC-WNL,P-Ad	32	34	0.6	18	0.6	31	92	1.0	✔	-	-	-	-	-	-	DF	Recovered
2.	-	+	13.6	38.1	4500	57.2	31.9	0.5	9.9	0.5	33000	NN,P-↓	23	32	8	36	1.1	32	73	1.0		✔	0	✔	2	3	-	DHF	Recovered
3.	-	+	13.1	32.2	7400	61.8	19.3	0.8	16	2.1	48000	NN,P-↓	86	101	2.1	45	1.9	38	433	1.0	✔	-	-	-	-	-	-	DF	Recovered
4.	-	+	12.8	34	5500	54.8	17.8	0.9	14.9	1.7	67000	NN,P-↓	38	68	0.3	22	0.8	29	167	1.0	✔	-	-	-	-	-	-	DF	Recovered
5.	-	+	12.4	32.8	2300	46.5	33.6	6.6	13.1	0.4	137000	NN,P-↓	86	104	1	17	1.3	31	103	1.0	✔	-	-	-	-	-	-	DF	Recovered
6.	+	+	17.4	51	5500	59.9	30.8	0.8	6.9	1.6	21000	NN,P-↓	160	227	0.7	35	0.9	38	144	1.07		✔	0	✔	5	6	-	DHF	Recovered
7.	-	+	12.1	29	2300	74	13.6	1.1	11	0.3	125000	NN,WBC-↓,P-↓	52	75	0.3	18	0.7	32	117	1.1		✔	3	✔	1	4	-	DF	Recovered
8.	+	+	14.2	34	3700	42	36	4.0	16	0.9	62000	NN,P-↓	128	214	1.1	28	0.8	36	114	1.2		✔	3	✔	1	2	-	DF	Recovered
9.	-	+	12.8	33	2800	47.4	37.2	0.8	13.4	1.2	84000	NN,WBC-↓,P-↓	56	58	0.9	19	0.7	31	122	1.0		✔	3	✔	1	3	-	DF	Recovered
10.	-	+	14.4	41.7	1400	40.4	49.0	1.2	7.6	1.8	56000	NN,WBC-↓,P-↓	182	265	0.5	17	0.7	34	341	1.0		✔	3	✔	2	4	-	DHF	Recovered
11.	-	+	11.8	41	3800	65.5	20.7	0.9	12.9	1.0	79000	NN,WBC-↓,P-↓	211	142	0.8	17	0.9	38	129	1.0		✔	2	✔	3	5	-	DHF	Recovered
12.	+	+	11.5	36	3300	60.7	31.4	1.9	3.9	2.1	41000	MH,WBC-↓.P-↓	40	32	0.4	36	0.9	30	126	1.0		✔	1	✔	1	3	-	DF	Recovered
13.	-	+	10.8	33	3600	66.9	15.0	1.0	16.1	1.0	34000	MH,WBC-↓.P-↓	85	278	1.4	34	1.3	32	142	1.0		✔	1	✔	2	3	-	DF	Recovered
14.	+	+	14	34	1500	66.5	21.3	1.9	9.8	0.5	31000	NN,WBC-↓,P-↓	73	32	0.8	25	0.9	32	99	1.0		✔	1	✔	1	3	-	DF	Recovered
15.	-	+	12.5	37	2600	54.5	32.6	0.7	10.8	1.4	34000	NN,WBC-↓,P-↓	24	157	1.0	17	1.0	40	158	1.0		✔	1	✔	1	3	-	DF	Recovered
16.	+	+	12.1	37.9	3600	33.8	48.4	0.5	11.8	5.5	60000	NN,WBC-↓,P-↓	48	29	0.8	34	0.7	28	127	1.0		✔	2	✔	1	3	-	DHF	Recovered
17.	+	+	10.9	32.0	6400	62.2	12.5	4.0	20.7	0.6	106000	NNA,WBC-N,P-↓	87	34	2.4	16	0.6	44	84	1.0	✔	-	-	-	-	-	-	DF	Recovered

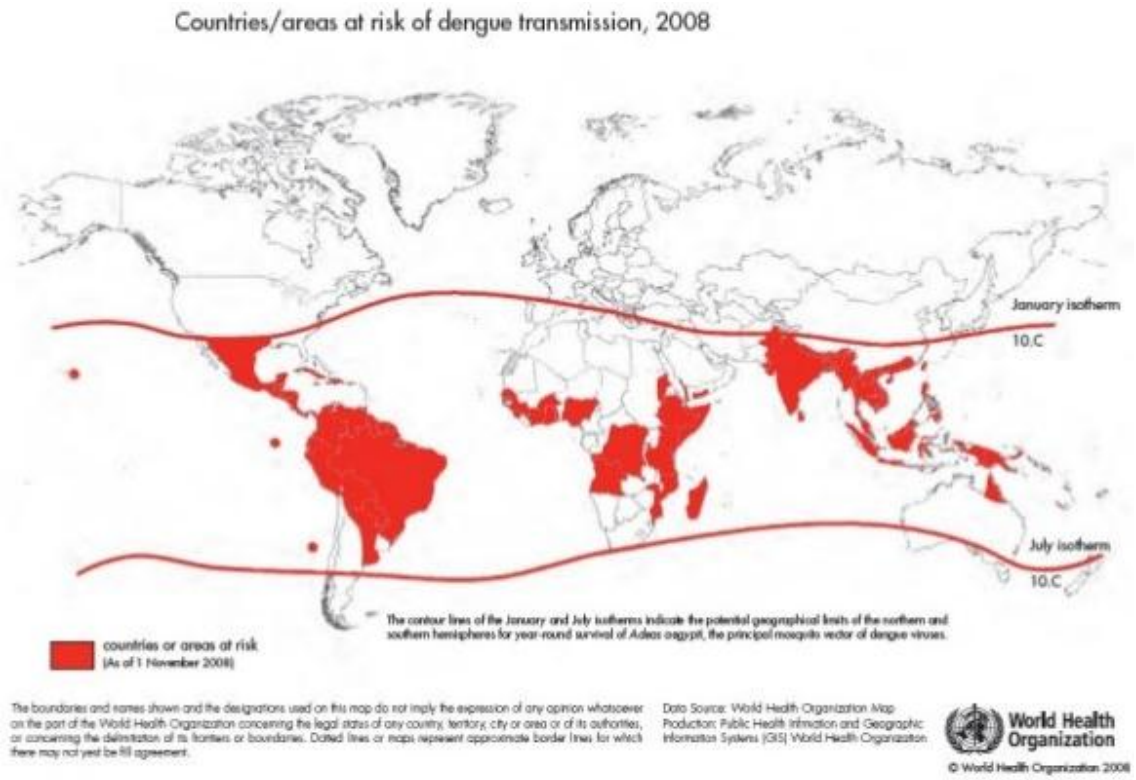
ANNEXURE-II (MASTER CHART)

Haematological Parameters												Biochemical parameters																				
Sl No.	NS1Antigen	IgM	Hb%	PCV	WBC	Differential Count						P.Smear	AST (SGOT)	ALT (SGPT)	S.Bilirubin	S.Urea	S.Creatinine	APTT	B.Glucose	PT INR	Not Done	Done							Profile	Final Outcome
						Poly	Lymph	Eosino	Monocytes	Baso	Platelet Count											Indication	Day of Initiation	Thrombocytopenia	No.of units	Day of Normal Platelet	Adverse effects		
18.	-	+	10.7	28	9600	48	34	0.8	14.8	2.3	10000	MHA,WBC-↓.P-↓	58	63	0.8	89	1.7	52	125	3.2		✔	1	✔	5	6	-	DHF	Recovered
19.	-	+	13.0	33.0	6200	63	22	3.8	9.5	1.4	19,000	NN,WBC-↓,P-↓	33	80	3.7	68	1.2	38	128	1.07		✔	1	✔	5	4	-	DHF	Recovered
20.	-	+	10.4	33.0	7500	32.9	52.2	0.6	11.8	0.8	1,23,000	MHA,WBC-N,P-↓	64	46	0.8	26	0.7	32	110	1.0		✔	3	✔	2	5	-	DHF	Recovered
21.	-	+	13.7	40.9	3300	50.9	31.7	1.5	15.5	0.9	27,000	NN,WBC-↓,P-↓	221	351	3.3	34	0.8	30	131	1.0		✔	1	✔	4	3	-	DF	Recovered
22.	-	+	15.0	27.6	6300	27.6	63.3	0.4	7.5	0.9	34,000	NN,WBC-N,P-↓	62	64	2.9	48	1.4	50	73	1.27		✔	1	✔	4	3	-	DHF	Recovered
23.	+	+	11.9	38.8	1200	58.9	30.1	1.7	6.4	2.9	25,000	NN,WBC-↓,P-↓	115	58	1.2	18	0.9	37	101	1.07		✔	1	✔	5	6	-	DHF	Recovered
24.	-	+	10.9	30.5	9000	53.8	32.0	0.4	12.1	1.7	41,000	NNA,WBC-N,P-↓	27	21	2.3	45	0.9	32	85	1.0		✔	1	✔	1	3	-	DF	Recovered
25.	-	+	14.3	39.7	4000	50.1	27.7	0.7	18.7	2.8	18,000	NN,WBC-N,P-↓	98	58	2.8	47	1.0	98	368	0.8		✔	1	✔	3	4	-	DHF	Recovered
26.	+	+	10.3	31.0	7700	59.9	19.9	3.3	7.1	0.3	98,000	MHA,WBC-N,P-↓	122	136	0.8	72	6.4	32	86	1.4		✔	1	✔	10	4	-	DHF	Recovered
27.	+	+	14.6	45	2900	40.5	30	12.8	15.7	0.8	35,000	NN,WBC-↓,P-↓	442	336	0.9	16	0.9	46	112	1.2		✔	1	✔	8	6	-	DHF	Recovered
28.	-	+	13.3	40	5700	49.7	36	2	10.4	0.7	34,000	NN,WBC-N,P-↓	22	29	1.2	36	0.6	37	131	1.2		✔	1	✔	2	3	-	DHF	Recovered
29.	-	+	13.2	36	10300	18	50	1	20	11	38,000	NN,WBC-N,P-↓	171	162	0.6	14	0.8	41	220	1.3		✔	1	✔	1	3	-	DF	Recovered
30.	+	+	14.8	53	4300	56.2	20.4	1.2	16.9	5.3	35,000	NN,WBC-N,P-↓	257	87	0.9	26	0.9	26	87	0.9		✔	1	✔	1	3	-	DF	Recovered
31.	+	+	14.9	48	3800	52	28.1	2.0	15.0	1.9	32,000	NN,WBC-↓,P-↓	124	319	0.9	13	0.4	39	103	1.2		✔	2	✔	1	3	-	DF	Recovered
32.	+	+	14.9	49.1	5300	74.1	14.1	3	8.3	0.5	1,01,000	NN,WBC-N,P-↓	134	125	1.2	20	0.7	30	140	1.0		✔	2	✔	1	3	-	DF	Recovered
33.	-	+	10.9	30.4	4600	48.3	39.7	0.8	9.8	1.3	32,000	NNA,WBC-N,P-↓	66	49	0.7	24	0.9	35	142	0.9		✔	2	✔	2	3	-	DF	Recovered
34.	-	+	12.4	32.8	2300	46.5	33.6	0.4	13.1	0.4	1,37,000	NN,WBC-↓,P-↓	86	104	1.0	17	1.3	31	103	1.0	✔		2	✔	2	2	-	DF	Recovered
35.	+	+	17.4	51	5500	59.9	30.8	0.8	6.91	1.6	21,000	NN,WBC-N,P-↓	160	227	0.7	35	0.98	38	144	1.0		✔	1	✔	5	6	-	DHF	Recovered

ANNEXURE-II (MASTER CHART)

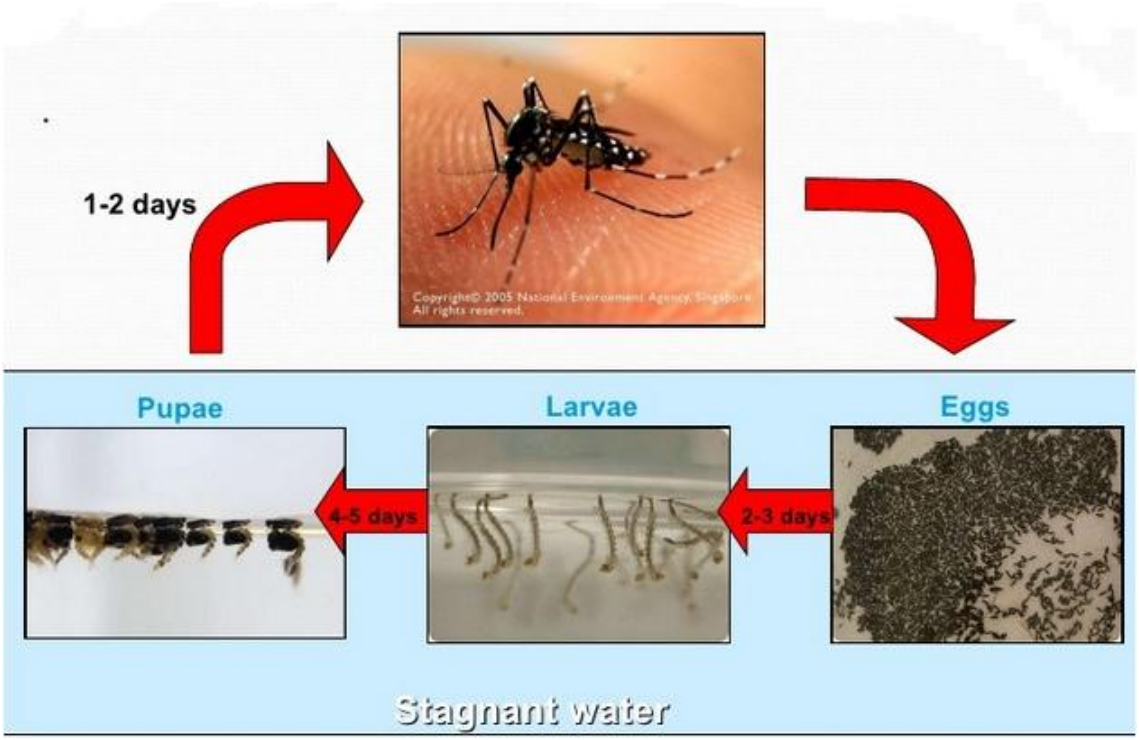
Haematological Parameters												Biochemical parameters																				
Sl No.	NS1Antigen	IgM	Hb%	PCV	WBC	Differential Count						P.Smear	AST (SGOT)	ALT (SGPT)	S.Bilirubin	S.Urea	S.Creatinine	APTT	B.Glucose	PT INR	Not Done	Done							Profile	Final Outcome
						Poly	Lymph	Eosino	Monocytes	Baso	Platelet Count											Indication	Day of Initiation	Thrombocytopenia	No.of units	Day of Normal Platelet	Adverse effects		
36.	-	+	12.1	29	2300	74	13.6	1.1	11	0.3	1,25,0000	NN,WBC-↓,P-↓	52	75	0.3	18	0.7	32	118	1.0		✔	3	✔	1	4	-	DF	Recovered
37.	+	+	12.5	37	2600	54.5	32.6	0.7	10.8	1.4	34,000	NN,WBC-↓,P-↓	224	157	1.0	17	1.0	40	158	1.0		✔	1	✔	1	3	-	DF	Recovered
38.	-	+	10.8	33	3600	66.9	15.0	1.0	16.1	1.0	34,000	MHA,WBC-↓,P-↓	85	278	1.1	34	1.3	32	142	1.0		✔	1	✔	2	4	-	DF	Recovered
39.	+	+	12.1	37.9	3600	33.8	48.4	0.5	77.8	5.5	60,000	NN,WBC-↓,P-↓	48	29	0.8	34	0.7	28	127	1.0		✔	2	✔	1	3	-	DHF	Recovered
40.	+	+	10.9	32	6400	62.2	12.5	4.0	20.7	0.6	1,60,000	MHA,WBC-N,P-N	87	34	2.4	16	0.6	44	84	1.0	✔	-	-	-	-	-	-	DF	Recovered
41.	-	+	10.7	28	9600	48	34	0.8	14.8	2.3	10,000	NNA,WBC-N,P-↓	58	63	0.8	89	1.7	52	125	3.2		✔	1	✔	5	2	-	DHF	Recovered
42.	-	+	13.0	33	6200	63	22	3.8	9.5	1.4	19,000	NN,WBC-N,P-↓	33	80	3.7	68	1.2	38	128	1.0		✔	1	✔	5	3	-	DHF	Recovered
43.	-	+	10.9	30.5	9000	53.8	32	0.4	12.1	1.7	41,000	MHA,WBC-N,P-↓	27	21	2.3	45	0.9	32	85	1.0		✔	1	✔	1	3	-	DF	Recovered
44.	-	+	14.3	39.7	4000	50.1	27.7	0.7	18.7	2.8	18,000	NN,WBC-N,P-↓	98	58	2.8	47	1.0	38	368	1.1		✔	1	✔	3	4	-	DF	Recovered
45.	+	+	10.1	31	7800	69.9	19.9	3.3	7.1	0.3	98,000	MHA,WBC-N,P-↓	122	136	0.8	72	6.4	32	86	1.1		✔	1	✔	8	4	-	DHF	Recovered
46.	+	+	14.0	42	2900	40.5	30	12.8	15.7	0.8	35,000	NN,WBC-↓,P-↓	422	336	0.9	16	0.9	46	112	1.2		✔	1	✔	6	5	-	DHF	Recovered
47.	-	+	13.3	40	5700	49.7	36	2	10.4	0.7	34,000	NN,WBC-N,P-↓	20	29	1.2	36	0.6	37	131	1.2		✔	1	✔	2	3	-	DF	Recovered
48.	-	+	13.2	36	10300	18	50	1	28	3	38,000	NN,WBC-N,P-↓	171	162	0.6	14	0.8	41	220	1.3		✔	1	✔	1	3	-	DF	Recovered
49.	+	+	14.2	34	3700	42	36.2	4.0	16	0.9	38,000	NN,WBC-↓,P-↓	128	214	1.1	28	0.8	36	114	1.2		✔	2	✔	1	3	-	DF	Recovered
50.	+	+	12.8	33	2800	47.4	37.2	0.8	13.4	1.2	84,000	NN,WBC-↓,P-↓	56	58	0.9	19	0.7	31	122	1.0		✔	3	✔	1	3	-	DF	Recovered

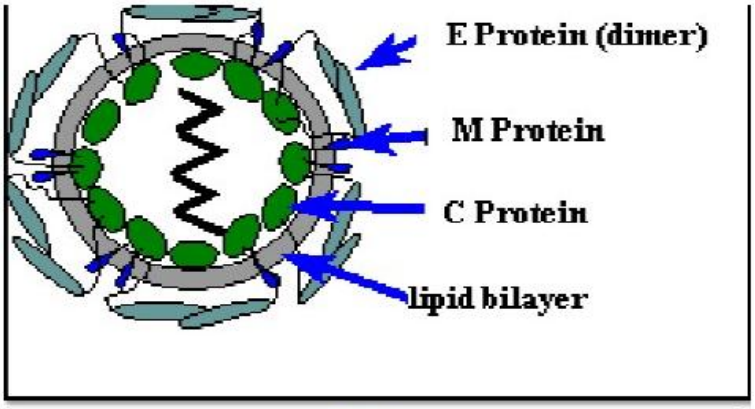
EPIDEMIOLOGY

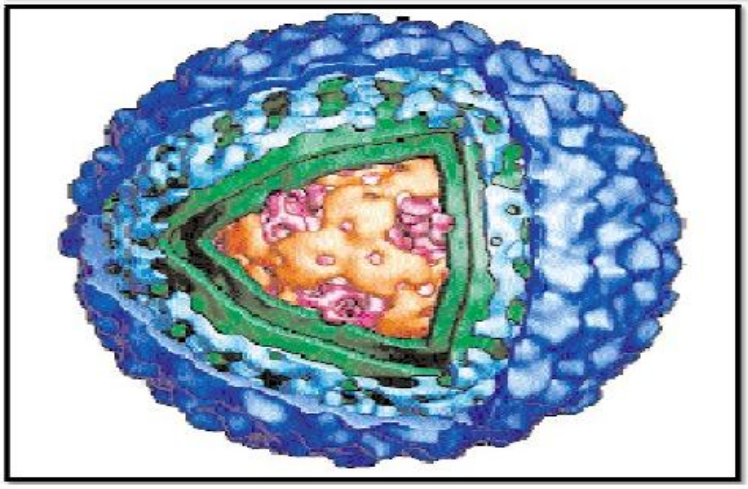


AEDES MOSQUITO

LIFE CYLE OF AEDES MOSQUITO

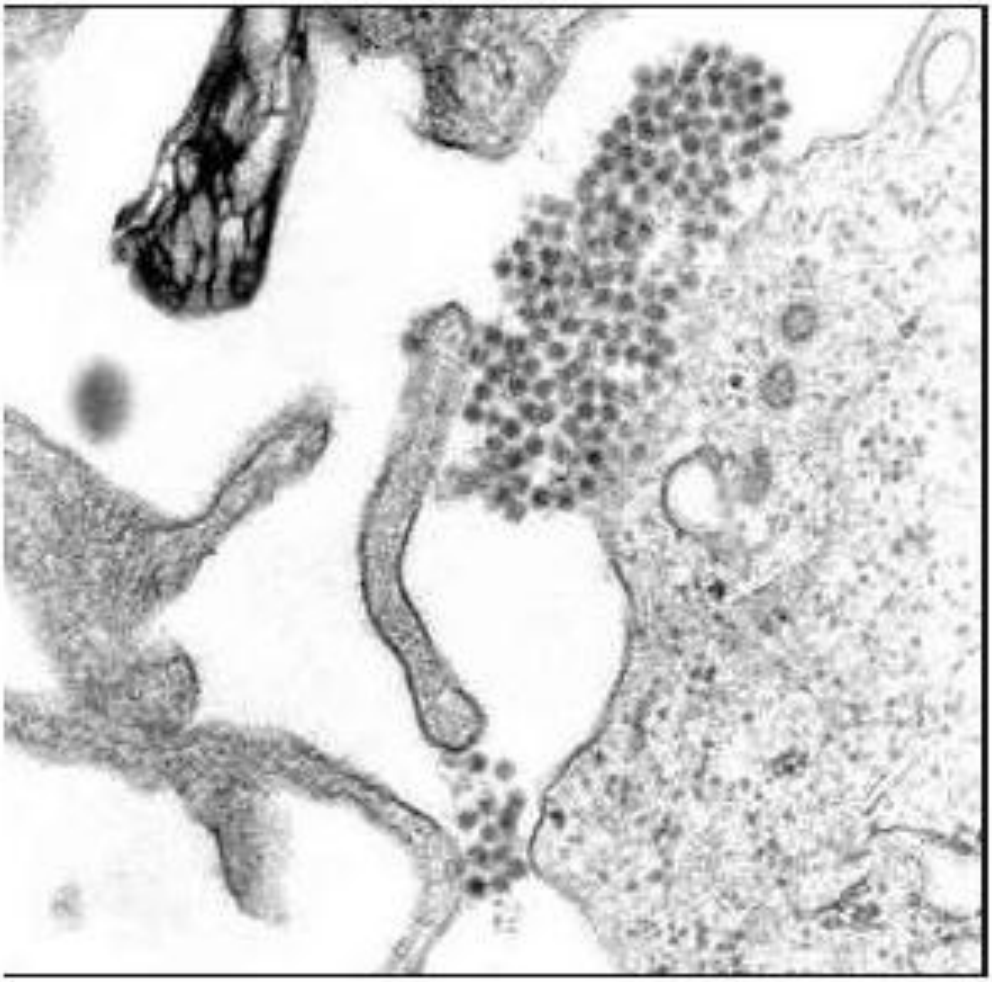






DENGUE VIRUS

ELECTON MICROSCOPY OF DENGUE VIRUS



WHO classification of dengue fever. (http://www.who.int/csr/resources/publications/dengue/Denguepublication/en/)

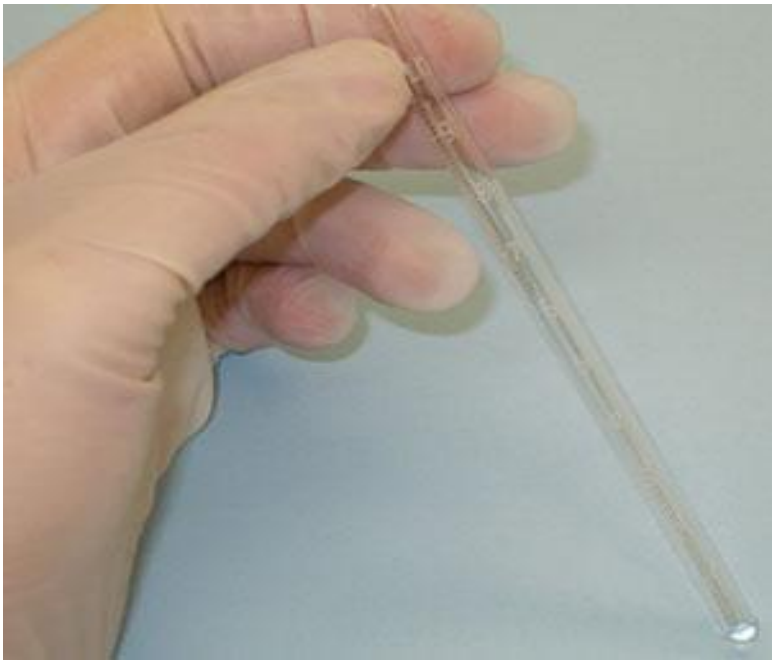
DF/DHF	Grade	Symptoms	Laboratory
DF		Fever with two or more of following: Headache Retro orbital pain Myalgias Arthralgias	Leucopenia, occasionally thrombocytopenia may be present. No e/o plasma loss.
DHF	I	Above signs plus positive tourniquet sign	Thrombocytopenia < 100 000; Hct rise ≥ 20%
DHF	II	Above signs plus spontaneous bleeding	Thrombocytopenia < 100 000; Hct rise ≥ 20%
DHF*	III	Above signs plus circulatory failure (weak pulse, hypotension, restlessness)	Thrombocytopenia < 100 000; Hct rise ≥ 20%
DHF*	IV	Profound shock with undetectable BP and pulse	Thrombocytopenia < 100 000; Hct rise ≥ 20%

WHO CLASSIFICATION OF DENGUE ILLNESS

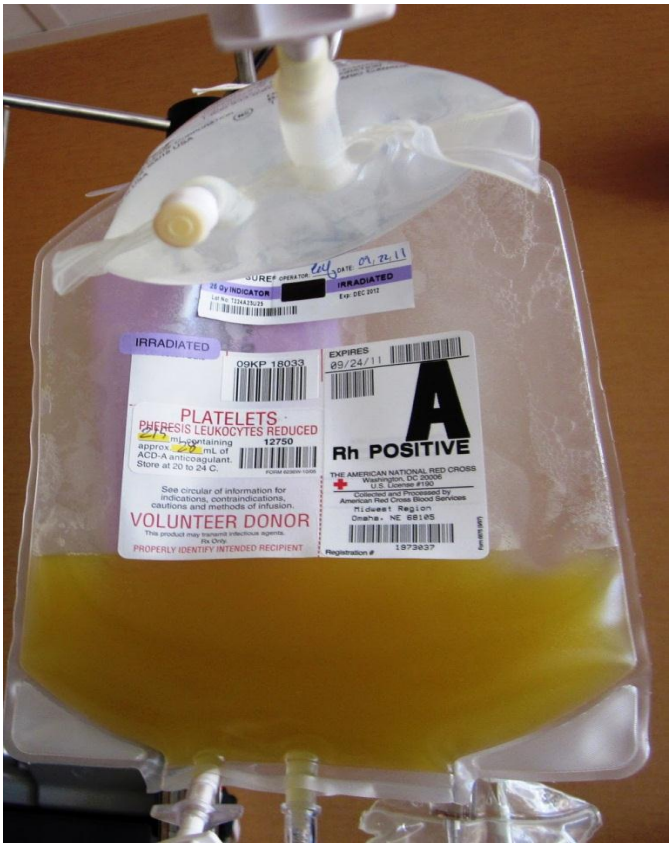
BECKMAN COULTER-AUTOMATED CELL COUNTER

REAGENTS FOR AUTOMATED CELL COUNTERS

T- COAG KC 1 DELTA

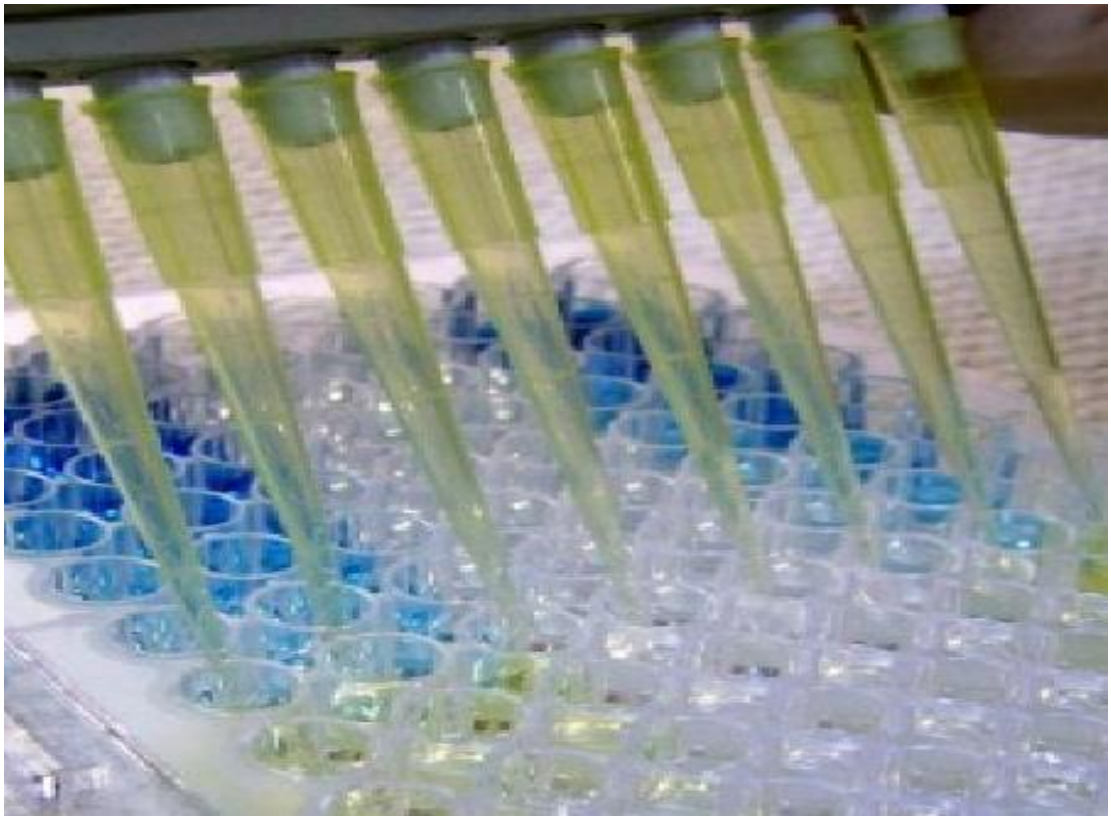


WINTROBES TUBE



PLATELET TANSFUSION

LEISHMANS STAIN

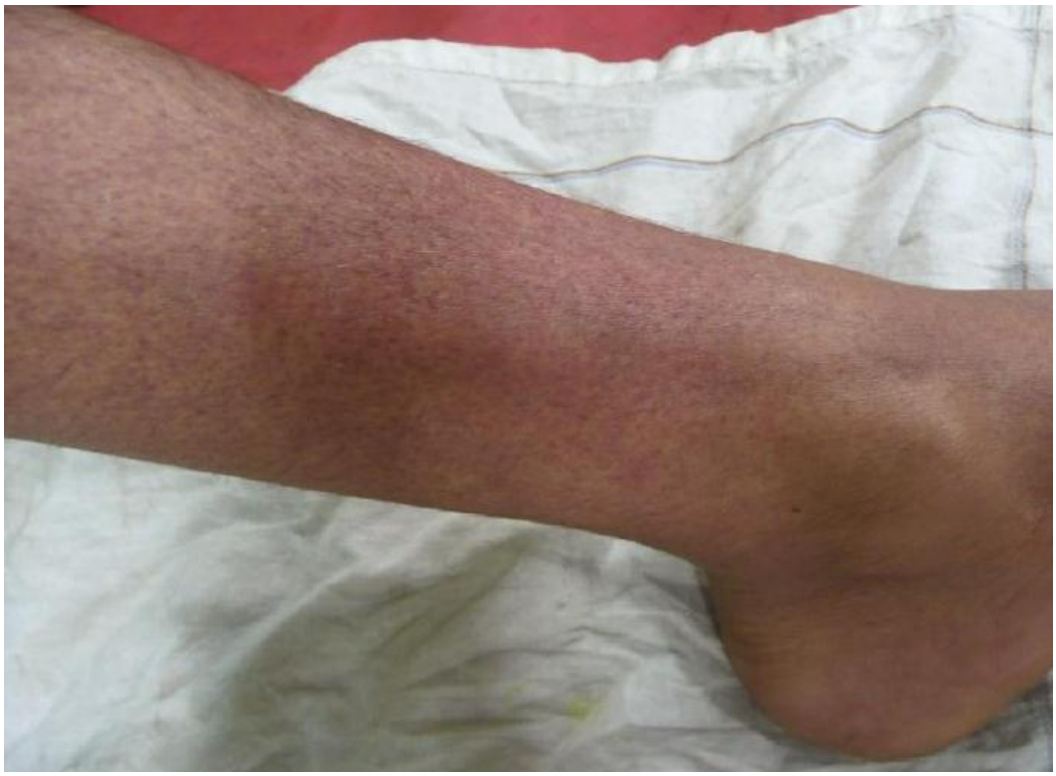


ELISA MICROWELLS

PAN-BIO ELISA KIT FOR IgM ELISA

EQUIPMENTS FOR ELISA

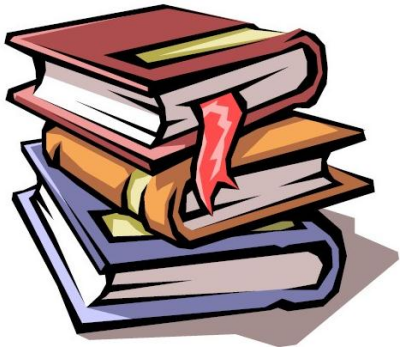
PETICHIAE ON THE LOWER EXTREMITIES



DF	-	DENGUE FEVER
DHF	-	DENGUE HAEMORRHAGIC FEVER
DSS	-	DENGUE SHOCK SYNDROME
NS 1	-	NON STRUCTURAL PROTEIN 1
WBC	-	WHITE BLOOD CELL
SEA	-	SOUTH EAST ASIA
TNF	-	TUMOUR NECROSIS FACTOR
WHO	-	WORLD HEALTH ORGANISATION
DALYS	-	DISABILITY ADJUSTED LIFE YEARS
CFR	-	CASE FATALITY RATE
DV	-	DENGUE VIRUS
RNA	-	RIBONUCLEIC ACID
RT-PCR	-	REVERSE TRANSCRIPTASE POLYMERASE CHAIN REACTION
ELISA	-	ENZYME LINKED IMMUNOSORBENT ASSAY
HI	-	HAEMAGGLUTINATION INHIBITION
CFT	-	COMPLEMENT FIXATION TEST
NT	-	NEUTRALIZATION TEST
GAGS	-	GYCOSAMINOGLYCANS
PCV	-	PACKED CELL VOLUME
aPTT	-	ACTIVATED PARTIAL THROMBOPLASTIN TIME
PT	-	PROTHROMBIN TIME
SGOT	-	SERUM GLUTAMIC OXALOACETIC TRANSAMINASE
SGPT	-	SERUM GLUTAMIC PYRUVATE TRANSAMINASE
MAC ELISA	-	IgM ANTIBODY CAPTURE ENZYME LINKED IMMUNOSORBENT ASSAY
RANTES	-	REGULATED ON ACTIVATION, NORMAL T CELL EXPRESSED AND SECRETED

INTRODUCTION

AIMS AND OBJECTIVES



REVIEW OF LITERATURE

METHODOLOGY

STATISTICS

RESULTS

DISCUSSION

CONCLUSION

SUMMARY

DENGUE PERIPHERAL SMEAR

BIBLIOGRAPHY

ANNEXURE-I

ANNEXURE-II