

MULTIVARIATE ANALYSIS OF DENGUE IN A TERTIARY CARE HOSPITAL



Dissertation submitted in
Partial Fulfillment of the regulations required for the award of
M.D DEGREE
In
Microbiology – Branch IV



THE TAMILNADU DR.M.G.R MEDICAL UNIVERSITY,
CHENNAI – 600 032.

April 2013

DECLARATION

I Dr P.Malini solemnly declare that the dissertation entitled “**Multivariate Analysis of Dengue in a Tertiary Care Hospital**” was done by me at Coimbatore Medical College, Coimbatore during the period September 2011 to August 2012 under the supervision and guidance of Dr.**K. RAJENDRAN BSc, M.D.**, Professor and HOD Department of Microbiology Coimbatore Medical College Coimbatore – 14.

This dissertation is submitted to the TamilNadu Dr.M.G.R Medical University, Chennai towards the partial fulfillment of the requirement for the award of M.D Degree (Branch – IV) in Microbiology to be held in April 2013.

I have not submitted this dissertation on any previous occasion to any university for the award of any degree.

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CERTIFICATE

This is to certify that the dissertation entitled “**MULTIVARIATE ANALYSIS OF DENGUE IN A TERTIARY CARE HOSPITAL**” is a bonafide work done by **Dr.P.Malini**, post graduate student in the Department of Microbiology, under the supervision of **Dr.K.RAJENDRAN B.Sc, M.D.**, Professor & Head Department of Microbiology, Coimbatore Medical College, Coimbatore, in fulfillment of the regulation of the Tamil Nadu Dr.M.G.R Medical University, towards the award of M.D Degree (Branch – IV) in Microbiology.

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CONTENTS

S.No.	Page No.
1. INTRODUCTION	1
2. AIM AND OBJECTIVES	4
3. REVIEW OF LITERATURE	5
4. MATERIALS AND METHODS	34
5. RESULTS	47
6. DISCUSSION	64
7. SUMMARY	78
8. CONCLUSION	82
9. BIBLIOGRAPHY	
APPENDICES	
(i) APPENDIX 1	
(ii) APPENDIX 2	
(iii) LIST OF TABLES	
(iv) LIST OF CHARTS	
(v) LIST OF COLOUR PLATES	
(vi) LIST OF ABBREVIATIONS	
(vii) PROFORMA	
(viii) PROTOCOL	
(ix) WORKSHEET	
(x) CONSENT FORM	
(xi) MASTER SHEET	

INTRODUCTION

Dengue virus is increasingly recognized as one of the world's major emerging infectious tropical diseases¹. According to WHO, Dengue fever or Dengue haemorrhagic fever is considered as the **second most important tropical disease next to malaria**². Dengue in recent years has become a major international health problem. Annually there are 100 million new dengue viral infections reported worldwide with 5 lakh cases of Dengue haemorrhagic fever (DHF) and Dengue shock syndrome (DSS). There are around 30000 deaths every year which is mostly among children^{2,3,4}. DHF/DSS is known as one of the leading causes of mortality and morbidity among school going children in Tropical and Subtropical countries⁵.

In India, dengue fever has been known for the past 2 centuries. In India the virus was first isolated in 1945. All the four serotypes are endemic in India^{6,7}. In South India all 4 serotypes of dengue were isolated in Vellore between 1956 & 1960. Later on it started to be reported from other parts of India.

Dengue virus is a single stranded RNA virus with four serotypes. They are DEN1, DEN2, DEN3 & DEN4. Infection with one serotype of Dengue does not confer cross protection against the other serotype. On subsequent infection it may lead to serious forms of disease like Dengue

haemorrhagic fever and Dengue shock syndrome through immunopathological enhancement^{8,7}.

Clinically Dengue infection causes a wide spectrum of illness ranging from undifferentiated dengue fever, Dengue haemorrhagic fever and Dengue shock syndrome which can finally lead to death⁷.

Commonly used diagnostic methods for Dengue are viral isolation, RT-PCR and serological methods. Viral isolation is a time consuming and fastidious process that requires specialized laboratory equipments and experienced personnel. RT-PCR even though significantly reduces the processing time and detects the virus in early stage these methods remain expensive and technically difficult particularly in laboratory settings of the developing world⁹.

Serological diagnosis of Dengue has many advantages like more flexibility, wide availability of reagents, low cost and requirement of less equipments⁷. One of the definite methods to diagnose early Dengue infection is to detect specific antigen which directly correlate with underlying viremia and pathogenesis of infection^{10,9}.

There is no specific treatment or vaccine for Dengue infection. Hence in view of its life threatening complications and increased mortality rate, it is imperative to have rapid and sensitive laboratory methods for early recognition of disease. This helps to identify cases,

initiate treatment at the earliest and to reduce the complications associated with it. Apart from serological parameters, haematological profile and biochemical tests are also helpful as diagnostic markers in the detection of Dengue infection.

In view of the increased occurrence of Dengue and its complications, the study is undertaken to look for seropositivity of Dengue among patients attending tertiary care hospital with categorisation and comparatively evaluate the performance of ELISA and rapid card in the detection of dengue antigen and antibody.

AIM AND OBJECTIVES

Aim: Multivariate analysis of Dengue in a tertiary care hospital.

Objectives of the study:

1. To study the significance of seropositivity of Dengue in a tertiary care hospital.
2. To differentiate serologically between primary and secondary dengue.
3. To detect Dengue NS1 antigen, Dengue IgM and IgG antibody by rapid card method and ELISA technique.
4. To compare and evaluate the efficacy of rapid card with immunocapture ELISA in the diagnosis of dengue infection.
5. To correlate Dengue clinically along with haematological and biochemical markers.

REVIEW OF LITERATURE

History:

- First description of dengue was given by Benjamin Rush in 1780¹¹.
- Mosquito borne transmission of infection by *Aedes aegypti* was demonstrated in 1903 by Graham, by Bancroft in 1906 and by Cleland et al in 1918¹².
- Viral etiology was demonstrated in 1906 by Bancroft.
- Dengue viral serotypes were discovered in 1944¹.
- Dengue haemorrhagic fever was described after worldwar –II.
- First epidemic of DHF was reported from patients with haemorrhagic disease during an epidemic in Manila in 1956.
- Sabin and his colleagues showed that virus strains from 3 geographical areas like Hawaii, New guinea and India were antigenically similar¹².
- The strain isolated from Hawaii was called as DENV-1.
- Strain isolated from NewGuinea was called as DENV-2.
- DENV-3 & DENV-4 were isolated later on during epidemics in Manila.

Epidemiology:

The first reported major epidemic was in 1779-80 in Asia, Africa and North America which indicated that these viruses and their mosquito vector had world wide distribution in tropics for more than 200 years⁷. Earliest record of Dengue was seen in Chinese encyclopedia of disease symptoms and remedies first published during the JIN dynasty and again in 992 in northern sung dynasty. The disease was called as “**water poison**” by the Chinese and was thought to be connected with flying insects associated with water¹².

Global pandemic of Dengue began in South East Asia .It was first reported from Batavia in Jakarta of Indonesia¹² and intensified after world war II. Outbreaks of dengue fever epidemics were documented sporadically with a long gap of 10-30 years. In Southeast Asia, epidemic dengue haemorrhagic fever was first reported in 1950s. In 1975, it became a leading cause of hospitalization and death among children in many countries, with a case fatality rate of 1-5%^{13,14}. After a long interval of 10-40 years second pandemic occurred in Asia in 1980 including China and Taiwan. Dengue viruses were reintroduced in the pacific regions after 25 years in the early 1970s¹².

Recent epidemic in Srilanka and India was associated with multiple serotypes but DEN-3 serotype was predominant and different from the previous occurrence. In other countries of Asia where DHF is endemic, the epidemics have become progressively larger in last 15 years.

Epidemics reported around the world:

- Chartertower,Australia-1897
- Beirut- 1910
- Taiwan -1916
- Greece- 1928
- Taiwan-1931¹²

Currently dengue fever causes more illness and death than any other Arboviral disease of humans.The incidence has increased over the last 50 years with 2.5 billion people now living in dengue endemic areas¹². 100 million cases are reported every year worldwide with 5 lakh cases of dengue haemorrhagic fever and dengue shock syndrome. Around 30,000 deaths /year are reported which is mostly among children of 1to 14 years of age¹³.

India: The encounter of Dengue in India is interesting and intriguing. The clinical entity of dengue fever has been known for the past two centuries. Initially it was restricted to east coast of India which later affected most parts of India. It has been endemic in several parts with

interspersed Epidemics. The first major Epidemic in India was reported from Madras in 1780. The dengue virus was first isolated in Calcutta in 1944. Isolation of serotypes 1&4 was in 1964 followed by serotype 3 in 1968. In 1998 Dengue virus subtype 3 of Serotype 3 arose from India and spread to the world¹⁵. Since then serotypes were isolated from Vellore, Pondicherry, Chennai, Mangalore, Kolkata, Assam, Lucknow, Delhi & Haryana. Major outbreak of dengue in India was during October 2002 to December 2003. Dengue seems to be an emerging disease in TamilNadu according to Moorthy et al 2009¹⁶ and in other parts of India where Dengue fever & Dengue haemorrhagic fever have occurred.

Epidemics Reported in India:

Calcutta-1963, Vishakapatnam-1964, Vellore -1968, Ajmeer-1969, Kanpur-1969, Delhi- 1970, Rajasthan- 1985, Delhi- 1996, Delhi -2003, 2006, 2010^{16,14}

Dengue on rise:

Increased incidence and emergence of epidemic DHF is due to

1. Ecological changes
2. Massive human movement during World War II
3. Economic development
4. Unplanned and uncontrolled population growth
5. Uncontrolled urbanization.

6. Air travel by humans.
7. Lack of effective mosquito control in dengue endemic areas^{17,18,12,19}
8. Sub standard housing and inadequate water supply, sewage and waste management systems²⁰.
9. Presence of all four serotypes with secondary infection of a different serotype in the Host^{21,22,17,12}.

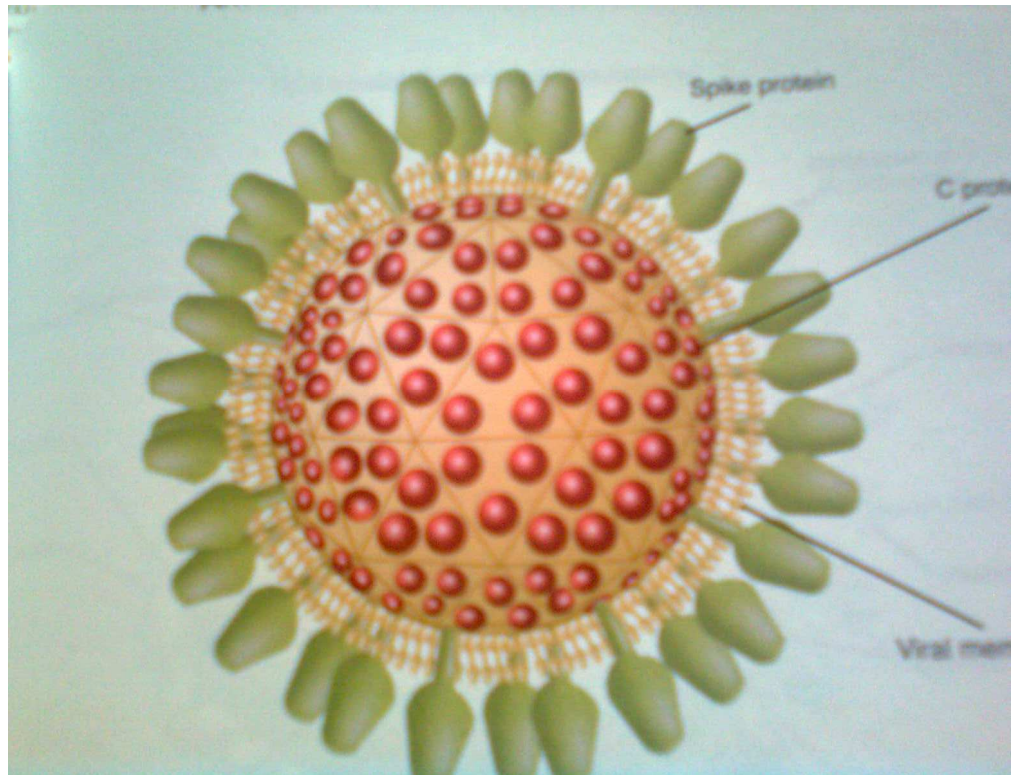
Dengue virus:

It is an enveloped positive sense ssRNA virus of 35- 40 nm diameter belonging to the Genus Flavivirus and family Flaviviridae. Genus has about 70 distinct viruses. Out of 70, 13 viruses are causing disease in humans and 3 are found to cause increased mortality.

Genomic structure:

The genomic RNA is approx. 11 kb in length. It is composed of 3 structural protein genes that encodes nucleocapsid or core protein , a membrane associated (m) protein ,envelope (e) protein and 7 non-structural proteins namely NS1,NS2a,NS2b,NS3,NS4a,NS4b,NS5. NS1 protein is secreted from virus infected cells¹⁹. NS1protein is involved in virion morphogenesis, NS2A and NS3 Protein has virus specific protease. NS3 has helicase activity. The nucleocapsid core is encapsulated by a lipid bilayer membrane.It has 180 copies of membrane proteins and 180

Dengue Virus



copies of envelope proteins which is anchored to it. DENV envelope is found to be the dominant antigen²³.

NS1 is 55KDa membrane bound glycoprotein whose function has not been fully determined. NS1 protein is responsible for inducing the soluble complement fixing antigen which is detected in virus infected cells .It is said to contribute to the pathogenesis of Dengue^{24,1}. In children elevated NS1 plasma concentration early in illness are associated with more severe disease reflecting high viral burden⁷.

Serotypes of Dengue:

There are 4 serotypes of dengue DEN-1, DEN-2, DEN-3&DEN-4 depending on antigenic and genetic characterization,^{19&16}. DEN 1 and 3 serotypes share antigenic determinants. Close relation between DEN1 and 4 serotypes has been proved by cDNA hybridization probe. DEN 2 serotype alone shows low sequence homology with all other serotypes.

Dengue epidemic results from introduction of new serotype in areas where already one serotype exists. Asymptomatic to symptomatic infection ratio in dengue viral infection varies with virus strain, age and immune status of the population.

Route of transmission:

Dengue fever is transmitted by the bite of the principal vector mosquitoes called *Aedes aegypti* followed by other secondary vectors

like *Aedes albopictus*, *Aedes scutellarius* & *Aedes polynesiensis*. In India the common vector is *Aedes aegypti*²⁵.

Aedes aegypti is an African mosquito. Slave trade between Africa and America and the resulting commerce were responsible for the introduction and widespread geographic distribution of *Aedes aegypti* in the new world during 17th, 18th & 19th centuries. It has black and white stripes on the body. *Aedes* mosquitoes are otherwise called as “**Tiger mosquitoes**”.

The life span of mosquitoes is increased by moderate temperature and increased humidity. There is a year round transmission between 35⁰N and 35⁰S with increased transmission during rainy season. With increasing spread of vector mosquitoes throughout the tropics and subtropics large areas of the world have been vulnerable to the introduction of dengue viruses through air travel by infected humans¹⁸.

Aedes Mosquitoes has a life span of 1 to 4 weeks. Following infection by dengue virus it requires an extrinsic incubation period of 1 to 2 weeks to become infective to humans. Even though infected it does not suffer from the disease. Sexual transmission occurs from male to female and not vice versa. Females mate only once in their lifetime and can produce eggs at intervals throughout their life. They require blood as the source of protein for maturation of eggs. Digestion of blood meal and

Vector – Female Aedes Aegypti



simultaneous development of eggs takes 2-3 days in the tropics but longer in temperate climates. Female mosquitoes lay 30 -300 eggs at a time.

Transovarian transmission⁷ in mosquitoes leads to propagation of virus to their progeny and it acts as reservoir for virus maintenance during inter-epidemic periods. Eggs are laid above the water surface and hatch only in water which can happen even in flower vases, water storage jars and rainwater collected in small cups, tyres etc^{26, 19}. If left dry they remain viable for many weeks.

Adult mosquitoes are indoor daytime biting mosquitoes. Females are said to be nervous feeders. Disruption of the feeding process of mosquitoes takes place even at the slightest movement of the biting person. Aedes will often feed on several persons for a single meal. During this single blood meal, if the mosquito is infective it may transmit dengue virus to multiple persons in a short period of time even if they only probe without taking blood. Because of this behavior it can affect all the family members which makes it an effective **Epidemic vector**^{12,19}. Mosquitoes are normally attracted by body odours, CO₂ and heat emitted from animals/human beings¹⁸.

Adult mosquitoes normally shelter indoors and bite at an interval 1-2 hrs particularly during early morning and late afternoon. The

commonest biting area preferred are over the ankles and exposed parts of neck.

Other modes of transmission:

Needle stick injuries¹.

Through blood transfusion in endemic areas during the viremic period^{27,28}.

Vertical transmission of dengue virus to neonates whose mothers had an onset of primary /secondary dengue fever upto 5 weeks before delivery has resulted in acute neonatal dengue manifesting as fever, apnoea, mottling, hepatomegaly and decreased platelet count¹.

Endemic transmission: In endemic countries 1/20 houses may contain infected mosquitoes¹.

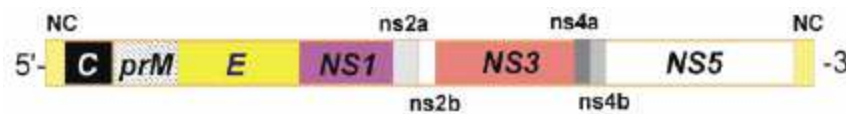
Viral replication:

DENGUE DISEASE

“A TICKING TIME BOMB OF HUGE EPIDEMIC POTENTIAL”

Following vector bite ,incoming genomic RNA serves directly as messenger RNA .It has a large open reading frame which is translated completely from its 5’ end to produce a large precursor polyprotein which is again cleaved to produce individual proteins. About 1/4th length of genomic RNA from 5’ end encodes 3 structural proteins and 3’ end encodes for non-structural proteins. Premembrane precursor protein is

cleaved and matures to form 'M' (membrane). NS3&NS5 proteins form RNA dependent RNA polymerase complex. NS3 is responsible for co-translational cleavage of nascent polyprotein that yields the NS protein whereas cellular signal peptidases affect other primary cleavage.



Replication occurs in the perinuclear foci leading to synthesis of complementary minus strand which is the template for synthesis of more positive strands during infection. Virion assembly takes place mainly on the endoplasmic reticular membrane in vertebrates and in plasma membrane in invertebrates. Fully formed cell is released usually by cell lysis.

Pathogenesis and Immunological reaction

After the bite of an infected mosquito, the virus replicates in local lymph nodes and within 2 to 3 days disseminates through the blood to tissues. Interstitial dendritic cells located in the epithelia are believed to constitute the first line of innate host immune response against invasion of dengue virus. Since phagocytic cells are serving as first line of defense, most of the infections are asymptomatic. Virus circulates in blood for 5

days in infected monocytes and macrophages and to lesser degree in B cells and T cells. It also replicates in the skin, spleen lymphoid cells and macrophages¹. Macrophage inflammatory protein-1 α has a pathogenic role in dengue virus infection⁸.

Infection of monocyte and macrophage is central to the pathogenesis of Dengue fever and origin of Dengue haemorrhagic fever or shock syndrome¹⁶. First infection with any of the dengue serotypes results in self limiting febrile illness. Recovery from first infection is usually accompanied by generation of immunological responses. Hence secondary infection with a heterotypic virus will result in severe illness. Epitopes present on Envelope protein are capable of inducing homologous as well as heterologous antibodies.

The plasma leakage is induced by C3a,C5a and several mediators leading to the increased vascular permeability during the acute febrile stage. The plasma leakage is prominent during “**critical period**” which lasts from 24 hrs before and 24 hours after the day of defervescence of fever¹. The evidence of plasma leakage includes hemoconcentration, hypoproteinemia/hypoalbuminemia, pleural effusion, ascites, threatened shock or profound shock⁷.

Vascular permeability in Dengue Haemorrhagic fever is associated with increased immune activation as manifested by increased levels of

plasma soluble TNF receptor (sTNFR/75) IL-8, IFN- γ , local endothelial proliferation of IL-8, RANTES with apoptotic endothelial cell death. Immune complex formed by antigen and antibody leads to complement activation¹.

The cause of bleeding diathesis in Dengue is complex. It is caused by vasculopathy, thrombocytopenia, platelet dysfunction and coagulopathy²⁹. Haemorrhagic diathesis is due to a combination of cytokine activation and vascular injury. Viral antibodies binding to platelets or cross reacting with plasminogen and other clotting factors, decreased platelet function and survival leads to mild consumptive coagulopathy.¹⁰

The pathogenesis of severe disease is not well understood and multiple factors may be implicated. During secondary infection with another serotype, the heterologous antibodies increases the viral uptake not neutralized and replication takes place in Fc receptor bearing cells. This phenomenon is called as **Antibody dependent enhancement (ADE)**^{30,31,29,12}. This forms the basis of severe dengue infection and in infants with primary infection. ADE- results in high viral load and increased inflammatory response which accounts for capillary leak syndrome⁶. From this it is clear that the dengue vaccine must induce protection simultaneously to all serotypes³².

Dengue haemorrhagic fever is an infrequent but potentially lethal form of illness usually due to secondary infection by heterologous serotype. The pathophysiology of DHF is multifactorial involving viral nature, host genetics, host immunity and previous exposure to the virus²⁹. Higher plasma viremia early in the course of infection and consequent T-cell activation predispose to DHF⁸.

In patients with DHF, production of various proinflammatory cytokines/chemokines and complement is increased. Genes encoding for IL-10, IL-8, IL-1 β , IL-32/NK4, IFN- γ , TNF- α , MIP-1 β , RANTES, CXCL7, CXCL1, properdin, and factor D component of complement were more strongly expressed in Peripheral blood mononuclear cells of patients with DHF than DF⁵.

DHF in primary or secondary dengue is due to the occurrence of abnormal immune response involving production of cytokines or activation of T-lymphocytes and disturbance of the hemostatic system²⁹. According to **Scott B Halstead**³³ DHF is not significantly associated with secondary dengue infection as it can result from a virulent dengue virus strain causing primary infection itself.

The mediators like C3a, C5a, TNF- α , interleukin (IL)-2, IL-6, IL-10, IFN- α and histamine are elevated in DHF than Dengue fever. This

indicates enhanced activation of cross reactive T cell which leads to increased viral uptake and replication in macrophages and monocytes. This is called as “**original antigenic sin**”²⁹.

Profound T cell activation and death of T cells, during acute dengue infection may suppress or delay viral elimination. This further leads to the higher viral loads and increased immunopathology found in patients with DHF²⁵.

Illness after infection with 2 serotypes occurs infrequently and illness after 3 infection virtually never. Repeated episodes of DHF have been recognized rarely, presumably because of immune factors that promote immunopathologic response are outweighed by immune response that clear infection.

Pathologic feature of Dengue in DHF/DSS-

Midzonal and hepatocellular necrosis, minimal inflammatory response, councilman bodies, microvesicular fatty changes, hypercellular and hyperplasia of mononuclear phagocytic cells in lymphoid tissue. Atypical lymphocytosis in peripheral blood, widespread infection of mononuclear phagocytes and endothelial cells are seen ¹². The degree of liver dysfunction in dengue infection varies from mild injury with elevation of aminotransferases alone to severe injury with jaundice and hepatic failure. The severity of dengue infection varies depending upon

type of infection and is more in complicated dengue. AST and ALT levels are increased in DHF¹

Malaise and flu like symptoms in Dengue are due to **cytokine response**. Myalgia is due to pathological changes in muscle due to moderate **perivascular mononuclear infiltrate with lipid accumulation**. Severe Musculoskeletal pain indicates viral infection of bone marrow including mobile macrophages and dendritic cells (CD11b/CD18 [Mac-1] positive) and relatively non motile adventitial reticular cells (nerve growth factor receptor positive).

Local suppression of erythrocytes, myelocytic and thrombocytopenic precursors within 4 – 5 days which is reflected in peripheral cytopenia resembling a picture of malignancy. Histopathologic examination of skin show minor degree of lymphocytic dermal vasculitis and variably viral antigen.

Thrombocytopenia and hemoconcentration are constant findings in DHF. A drop in platelet count below 1lakh is seen from 3rd day to 8th day of illness. Hemoconcentration with rise in hematocrit >20% is definitive evidence of plasma leakage. WBC count varies ranging from leucopenia to leucocytosis which is more common towards the end of febrile phase of illness.

Türk cells are transformed lymphocytes. Presence of more than 20 % of Türk cells in buffy coat smear is a frequent finding of dengue hemorrhagic fever³⁴.

Immunological Response to Dengue infection:

The acquired immune response following a dengue infection consists of the production of IgM and IgG antibodies primarily directed against the virus envelope proteins. The immune response differs for primary and secondary infection.

In primary dengue IgM is the first immunoglobulin isotype to appear. IgG is detectable in low titre at the end of first week and slowly increases. In contrast, in secondary dengue antibody titres rise extremely rapidly due to the presence of antibody to the previous infection and these antibodies react broadly with other flaviviruses. High levels of IgG are detectable even in acute phase and rise dramatically over the proceeding 2 weeks. IgM levels are significantly lower in secondary infections. PAHO says that 80% of all dengue cases have IgM antibody by day 5 of illness and 93-99% cases are detectable by 6-10 days and remain detectable upto 90 days. The presence of antibodies in dengue is protective to the infecting serotype but short lived for heterologous serotype. The level of neutralizing antibodies corresponds with protection against dengue virus.

Clinical features:

Incubation period of Dengue is 2 to 7 days and is characterized by high fever, headache, retro orbital pain, lumbosacral pain, conjunctival congestion and facial flushing. Biphasic fever pattern- fever upto 3rd day of onset which subsides and rises again from 5th to 7th day. Because of regular fluctuations in temperature it is also called as '**saddle back**' fever.

Fever is usually associated with severe generalized myalgia and arthralgia which gives the name as '**Breakbone fever**'. Maculopapular rash appears on the trunk on 1st /2nd day (non irritating rash) then spreads centripetally to the face and limbs but spares palms and soles. Generalised lymphadenopathy, cutaneous hyperaesthesia, altered taste sensation can occur.

Unusual manifestations are neurological, hepatic, renal and isolated organ involvement.

According to WHO Guidelines Dengue fever, Dengue haemorrhagic fever and Dengue shock syndrome are defined as follows:

Dengue fever: fever of 2-7 days associated with symptoms like Arthralgia, myalgia, vomiting, abdominal pain, rash, retro orbital pain, conjunctival congestion, haemorrhagic manifestation, or leucopenia.

Dengue haemorrhagic fever: DHF is defined by the presence of above symptoms plus thrombocytopenia, evidence of increased vascular

permeability (i.e.hemoconcentration, pleural or abdominal effusion, hypoalbuminemia or hypoproteinemia). DHF has 4 grades.

Dengue shock syndrome: Defined by the presence of symptoms of DHF with reduced perfusion towards the defervescence and early signs of shock manifested as narrowing of pulse pressure or hypotension for age.

The symptoms of DSS are cold clammy extremities, flushed face, diaphoresis, restlessness, irritability and mild epigastric pain¹².

Induction of shock in Dengue is influenced by the following:

1. Presence of enhancing and non- reacting antibodies.
2. DHF/DSS is common upto 12 years of age and drops later.
3. Females are affected more than males.
4. Race- Caucasians are affected more.
5. Nutritional status- Malnutrition is said to be protective as they have deficient immune response to infection.
6. Sequence of infection- Serotype 1 followed by Serotype 2 infection is more dangerous.
7. Infecting serotype- Type 2 is apparently more dangerous than other serotypes.

Period of shock is about 1-2 days¹⁸ so prompt supportive management and good care with close monitoring during this period would prevent complications and save the life of the patient .

- Apart from the above causes, WHO also defines the high risk group as infants and elderly, obesity, Pregnancy, peptic ulcer disease, women who have menstrual / abnormal vaginal bleeding. Other risk factors like G-6PD deficiency, thalassemia and hemoglobinopathies, chronic disease like DM, asthma, SHT are also said to be associated.³⁵

Laboratory Diagnosis:

Efficient and accurate diagnosis of dengue is of primary importance in detection of cases and early treatment. Laboratory diagnostic methods of detection of dengue are

Serological methods

Viral isolation

Detection of viral nucleic acid.

In **complete blood count** platelet count and the total WBC count would be reduced depending upon the type of fever. As the WBC count becomes low, platelet counts also become very low but during the recovery stage WBC count rises rapidly than Platelet count. Platelet count is usually reduced more during the DHF and DSS.

Neutropenia with atypical lymphocytosis resembling malignancy could be seen in peripheral smear. Packed cell volume should be estimated as it rises in Dengue haemorrhagic fever rapidly.

In **liver function test** –Enzymes like Aspartate aminotransferase and Alanine aminotransferase are elevated depending upon the type of injury.

Serological methods:

Rapid diagnostic tests:

Various commercial kits are available for dengue detection using the envelope glycoproteins of dengue for IgM and IgG antibody detection and NS1 antibody for antigen detection.

Lateral flow immunochromatographic test allows detection of both antigen and antibody simultaneously with single serum sample, therefore differentiation of primary and secondary dengue can be made with single sample in contrast to multiple samples required for HAI. It produces results within 15- 20 minutes and it has the advantage of ease of performance also. But the sensitivity and specificity of these tests are not known and are yet to be evaluated .According to WHO , these RDTs are not be used for clinical management because of false negativity that can lead to missing of diagnosis and fatal complications³⁶.

Enzyme immunosorbent assay (ELISA):

Antigen detection:

Enzyme Immunoassays for antigen detection have four steps. An antigen specific antibody is attached to a solid phase surface. Patient serum that may contain the antigen is added next. An enzyme labeled

antibody specific to the antigen (conjugate) is added. Finally substrate is added which changes colour in the presence of the enzyme. The amount of colour that develops is proportional to the amount of antigen present in the patient specimen.

NS1 protein can be detected by ELISA as soon as first day of fever and can be found upto 9 days in serum even when PCR is negative and where PCR is not available. It might serve as early prognostic marker for severe dengue infections³⁷.

Antibody detection:

Immunocapture ELISA: It is designed to detect a specific type of antibody such as immunoglobulin M or immunoglobulin G. Antibody specific for IgM or IgG is attached to solid phase surface. The Patient specimen potentially containing IgM or IgG is added. Specific antigen is added. Finally chromogenic substrate is added which in the presence of the enzyme changes colour. The amount of colour that develops is proportional to the amount of antigen specific IgM or IgG in the patient serum.

Capture ELISA is found to be highly sensitive and is routinely used in diagnosis of dengue infections. They are simple to perform and large number of samples can be tested at the same time.

A.IgM capture: It is the widely used ELISA for detection of antibodies. It shows good sensitivity and specificity only when used 5 or more days after onset of fever. A positive result indicates recent infection but cannot identify the serotype. There are different formats of capture ELISA available like capture ultramicro ELISA, dot ELISA, Auidot IgM capture ELISA and dipstick. Samples like saliva, serum on filter paper could also be used. Different commercial kits are available with varying sensitivity and specificity³⁸.

B.IgG capture: This capture ELISA is commonly used for classifying cases based on type of infection like primary and secondary infection. When used along with IgM the ratio is calculated and according to standard criteria it is classified as primary or secondary. In primary dengue IgG is detected after 2 weeks and persists for life. In secondary dengue IgG antibody to the previous infection rises immediately within 1-2 days before the appearance of IgM. It is followed by IgM to the present infection and IgG after 1 week²⁵.

Other serological methods available for the detection of dengue antibodies:

Neutralisation tests: These tests are technically demanding and time consuming and are performed only in reference laboratories²⁵

Complement fixation test: One of the classic methods for demonstrating

the presence of antibody in a patient's serum has been the complement fixation test. As the procedure is cumbersome, requires highly trained personnel and the reagents are thermolabile it is not used for routine diagnosis³⁹.

Heamagglutination inhibition test: This test can be used for suspected DHF/DSS but results are delayed in time when compared to Capture ELISA .Hence it cannot be used for routine diagnostic purposes⁴⁰.

Indirect immunofluorescence test⁴¹

Dot Blot ELISA⁴²

Viral isolation:

Mosquito Cell lines, mammalian cell lines and adult mosquito inoculation.

In acute phase of infection isolation and identification of the virus is the only way to diagnose early dengue infection. Serum collected from patient is applied to mosquito cell lines .After amplification of virus in cell line the serotype is identified using monoclonal antibodies. This technique is sensitive only if there is high viremia in the early stages. common cell lines used are C6/36(Aedesaegypti), AP61(pseudoscutellarius) The CPE on cell lines appears between 1-4 days post inoculation –cells become round and swollen leading to multinucleated giant cells and syncytia formation.

In Mammalian cell culture (LLC-MK2) –CPE occurs after 14 days.

Least sensitive methods are Intra thoracic inoculation of mosquitoes.

Eventhough viral culture once considered as "Goldstandard", because of its lower sensitivity, long time required and applicable only for acute samples this procedure has been replaced by molecular diagnosis⁴³.

Animal inoculation: Intracerebral inoculation of serum into suckling mice of 1-2 days old done using tuberculin syringe into the lateral hemisphere. After inoculation the mice is observed for sickness daily for any sign of abnormal behavior and if positive it is euthanised, brain tissue is harvested and subjected to relevant tests for confirmation. As this is a tedious procedure and time consuming, it cannot be applied for routine use⁴³.

Detection of Nucleic acid: RT –PCR provides a rapid and simple method for detection of RNA viruses in serum samples and tissue specimens. Viral genome is amplified using oligonucleotide primers and the amplified product is detected using reverse transcriptase enzyme. Recently conventional RT-PCR has been replaced by Real time PCR which can be automated. Sensitivity ,Specificity and rapid detection of minute quantities of nucleic acid in patient's serum makes RealTime - PCR more sensitive with less contamination and easy identification of circulating serotype by analyzing the unique sizes of amplicon. Other

methods available for nucleic acid detection are NASBA, Hybridization probes⁴⁴.

Differential diagnosis of Dengue fever: Scrub typhus, Leptospirosis, Malaria, Chikungunya, Influenza, Measles, Rubella⁴⁵. These diseases could be ruled out by clinical examination and appropriate laboratory tests

Dengue vaccine: More researches have been carried out to develop a dengue vaccine that is safe and immunogenic against all 4 serotypes. Attempts to develop an effective vaccine have been hampered by the lack of understanding of the pathogenesis of disease and absence of suitable experimental model. Dengue vaccine must provide solid and long lasting protection against all dengue virus serotypes. Live attenuated tetravalent candidate vaccine are in the late stage of development which is produced by serial passage of wild type virus in primary dog kidney cells or other cell types⁴⁶. Sanofi Pasteur has reported successful results of phase II trials of tetravalent recombinant live attenuated vaccine⁴⁷.

Non- recombinant candidate vaccine using structural & non-structural purified proteins and synthetic peptides¹³

Recombinant subunit (Escherichia coli, baculovirus, yeast)¹³

Recombinant subunit vaccine was produced by coating the β domain of dengue serotypes 1-4 to the binding protein of E.coli. Its efficacy was

evaluated in mice as single or tetravalent vaccine. Neutralising antibody titres to each individual serotype was significantly greater than cross reactive neutralising titre compared to tetravalent vaccine⁴⁸.

Recombinant vector¹³

Infectious cDNA clone technology has also been exploited for development of dengue vaccine. A chimeric Yellow fever dengue type - 2 vaccine prepared by using recombinant cDNA of a Yellow fever vaccine strain as a backbone to which pre membrane and envelope gene of DEN-2 were inserted. It is in Phase II-III trial¹².

DNA vaccine is another novel and promising immunization approach. In order to improve the immunogenicity of DEN-2 candidate vaccine cytoplasmic region of envelope gene was replaced by lysosome associated membrane protein.¹³.

Treatment: No specific drugs are available for the treatment of Dengue. Proper maintenance of fluid balance is the cornerstone of management in Dengue.

Supportive and symptomatic treatment

- I.V. Fluids ,Bed rest
- Paracetamol for control of fever
- Aspirin and NSAIDS must be avoided.

- Platelet and HCT should be monitored daily beginning on the third day of fever.
- Blood and platelet transfusion if required –depending upon the occurrence of bleeding in GI tract but does not correlate with platelet count.
- Early recognition of leakage phase with prompt resuscitation and Close monitoring with oxygen, pulse, blood pressure and urine output would help to reduce complications and improves the treatment outcome.

Prevention: Neither vaccine nor specific drugs are available .Hence prevention by vector control of *Aedes aegypti* plays a major role in Dengue endemic countries.

Vector control:

Mosquitoes that rest indoors are easy to control. Female *Aedes aegypti* are indoor daytime biters. It is difficult to avoid species biting in daytime than those species that bite at night.

Removal or filling of breeding habitats in man made & natural containers, burning of organic waste, screen fitting of mosquito proof lids to drinking water storage containers, installing piped drinking water supply.

Safe and effective larvicides to breeding sites.

Personal protective equipments for the daytime biting mosquitoes are protective clothing like wearing long sleeved clothes, repellents & house screening ,indoor space spraying ,mosquito coils, mats, bed nets & air conditioning.

Applying insect repellents to both skin and clothing using permethrin is found to be effective. The most effective repellents contain DEET.

Biological control

Antilarval measures:

1. Eliminating or changing the breeding place to make it unsuitable for development of larvae.
2. Making the breeding place inaccessible to adult mosquitoes.
3. Releasing fish/predators that feed on larvae.

Gambusia affinis /*Poecilia reticulata* endotoxin producing bacteria (*Bacillus thuringiensis* serotype H-14, *Bacillus sphaeria*) and copepod crustaceans are currently used .These are suitable for large containers that are not cleaned regularly as frequent cleaning leads to depletion of nutrients available for the larvae.

4. Applying larvicides wherever necessary.

Antiadult measures:

1. Epidemic situations- outdoor space spraying with insecticides.
2. Insecticidal spray are usually applied to parts of town where

Abundant breeding sites are available supporting large
Population of Aedes.

3. Pyrethrin Knockdown sprays /Organo phosphate sprays can be
delivered in microdroplets^{49,26}.

MATERIALS AND METHODS

Study design: This is a Prospective study

Study period: One Year from September 2011 to August 2012. Approval from Ethical committee obtained for this study.

Inclusion criteria: Patients suffering from fever of 1-12 days duration with any of these symptoms like myalgia, arthralgia, headache, rash, anorexia, nausea, and vomiting, abdominal pain.

Exclusion criteria: Patients suffering from other nonspecific fevers without clinical features suggestive of dengue fever, Urinary tract infections, pneumonia and lung abscess etc.

Statistical analysis: Statistical analysis of results of this study was done using SPSS version 17, p value obtained from chi-square test.

Sample collection: About 5 ml of venous blood was drawn from 350 suspected patients of dengue fever after taking informed consent, under aseptic precautions in sterile containers. These samples were centrifuged at 1500 rpm for 10 minutes & serum separated. If the sample was not tested immediately it was stored at -20°C .

Methodology

The serum samples collected were subjected to Dengue duo NS1Ag +Ab combo cassette, NS1 capture ELISA, IgM Capture and IgG Capture ELISA in the Department of Microbiology, at Coimbatore medical college hospital.

Immunochromatography card test (ICT):

Dengue duo combo card:

The rapid test is an in-vitro immunochromatographic one step assay to detect both dengue virus Nonstructural (NS1) antigen and differential IgG/IgM antibodies to dengue virus in serum, plasma or whole blood.

The left side of Dengue duocard is the NS1 Ag rapid test for qualitative determination of antigen. It has a circular well for addition of sample. The test device contains a membrane strip precoated with anti-dengue NS1 Ag capture on test band region.

The right side of the card is a solid phase immunochromatographic assay for rapid, qualitative and differential detection of IgG and IgM antibodies to dengue virus in serum, plasma and whole blood .The card has a square well for sample addition, a circular well for buffer and a lateral flow

membrane with colloidal gold conjugate containing recombinant dengue antigen and a control.

Principle of the test:

The dengue NS1 antigen card can identify dengue virus NS1 antigen in serum, plasma or whole blood specimens with a high degree of sensitivity and specificity. Dengue IgG/IgM rapid test is designed to simultaneously detect and differentiate IgG, IgM antibodies to dengue virus. This test can also detect all 4 serotypes by using a mixture of recombinant dengue envelope proteins.

When a specimen is added to the sample well anti-dengue IgG & IgM antibodies in the specimen will react with recombinant dengue envelope proteins –colloidal gold conjugates and forms a complex of antibody-antigen. As this complex migrates along the length of test device by capillary action, it will be captured by the relevant anti-human IgG and /or anti –human IgM immobilized in 2 test lines across the test device and generate a coloured line.

Procedure:

1. The card was placed on an even surface after being unwrapped from the cover. Id no & date noted down on the card

2. With the dropper provided 10 µl of serum was added to the sample well followed by 4 drops of buffer on the right side of the card.
3. With another dropper provided 100µl of sample was added to the antigen well on the left side of the card.
4. Results were read after 15-20 minutes.

Results:

Antibody

1. Appearance of bands in all the lines on the antibody card
2. Appearance of control & “M” line alone
3. Appearance of control & ”G” line alone.
4. Appearance of control line alone

Antigen:

Appearance of bands in control area alone

Appearance of bands in control and test regions.

Interpretation:

1. Antibody :

- a. Presence of control line alone- test is negative for antibodies.
- b. Presence of all the lines control, M and G – both IgM &IgG
Positive -Indicates either late primary or early secondary infection
- c. Presence of control and M line alone- primary dengue
- d. Presence of control and G line alone- secondary or past dengue

e. Absences of control line – invalid and test to be repeated.

2. Antigen :

a. Presence of bands in both test & control area – positive for NS1 antigen.

b. Presence of band in control area alone- negative for antigen

c. No bands in the result window, it is invalid– test has to be repeated.

Dengue EARLY ELISA

Principle of the test: NS1 antigen present in the serum binds to the anti-NS1 coated on the microwell strips. The unbound antigen if present is removed by the washing step. HRP conjugated monoclonal anti-NS1 antibody is added. A colourless substrate TMB is added which produces blue colour. On addition of stop solution phosphoric acid it changes to yellow colour.

Procedure:

1. 100µl of diluted sample & controls were added into the respective wells
2. The plate was Covered & incubated for 1 hour at 37⁰ c
3. At the end of 1 hour Plate was washed 6 times with diluted wash buffer

4. 100µl of Horseradish peroxidase(HRP) conjugate was added into the well.
5. The plate was Covered & incubated at 37⁰ c for 1 hour.
6. Washed 6 times with diluted wash buffer at the end of incubation.
7. 100µl of Tetramethylbenzidine(TMB) was added into each well
8. Incubated for 10 mts at Roomtemperature(RT) & observed for blue colour
9. 100µl of stop solution was added & the change of colour noted.
10. Absorbance value of each well measured at 450 nm with reference filter 600-650nm spectrophotometrically.

Calculation:

Average absorbance of calibrator triplicate multiplied by the calibration factor

Cut off value(COV) = absorbance of calibrator X cal. Factor

Cal .factor=0.57

Index value= sample OD/ cutoff value

Panbio units= Index value x 10

Test validity:

Negative absorbance < 0.250

COV >1.5 X Negative absorbance.

Positive control/ cutoff ratio= 1.1-7.0

Interpretation:

Index	Panbiounits	Result
<0.9	< 9	Negative
0.9-1.1	9-11	Equivocal
>1.1	> 11	Positive

Dengue IgM capture ELISA:**Principle of the test:**

Serum antibodies of the IgM class, when present, combine with anti-human IgM antibodies coated on the polystyrene surface of the microwell test strips (assay plate). The diluted recombinant antigen is mixed with equal volume of horseradish peroxidase (HRP) conjugated monoclonal antibody (MAb) allowing the formation of antigen-MAb complexes. Addition of complexed antigen-MAb complexes following washing will bind to the serum dengue-specific IgM antibodies. After incubation a colourless substrate tetramethylbenzidine/hydrogen peroxide

(TMB Chromogen) is added. The substrate is hydrolysed by HRP if present, and the chromogen turns blue. After stopping the reaction with acid, the TMB turns yellow. Colour development is indicative of the presence of anti-dengue IgG antibodies in the test sample.

Procedure:

All the kit reagents were brought to room temperature before start of the test.

ELISA Procedure:

1. Dispensed 100µl of diluted patient serum, positive and negative control, calibrator in triplicate added to their respective wells in the microtiter plate.
2. The microtiter plate was covered and incubated at 37⁰ c for 1 hour
3. Antigen was diluted in 1/250 with antigen diluent i.e 10µl of antigen in 2.5ml of antigen diluent.
4. Required amount of diluted antigen is mixed with equal volume of Monoclonal antibody tracer and incubated at room temperature until required.
5. The plate was washed 6 times with diluted wash buffer at the end of incubation.

6. Added 100µl of antigen antibody mixture to all the wells
7. The plate was covered and incubated at 37⁰ c for 1 hour.
8. At the end of incubation, plate was washed 6 times with diluted wash buffer
9. Added 100µl of TMB substrate to the wells and incubated in the dark for 10 minutes.
10. 100µl of stop solution added to all the wells.
11. Absorbance value of each well measured spectrophotometrically at 450nm wavelength with reference filter of 600-650nm.

Calculation:

- Average absorbance of the triplicate of the calibrator multiplied by the calibration factor. This is the cutoff-value.
- An index value is calculated by dividing the sample absorbance by the cut-off value

Index value= sample absorbance/ cut-off value

Panbio units= Indexvalue x 10

Test validity:

Cutoff value > 1.5x neg. absorbance

Calibrator mean > negative absorbance

Positive control/cutoff value = 1.1-8

Negative control < 0.40

Calibration factor = 0.99

Interpretation of results:

INDEX	PANBIOUNITS	RESULT
0.9	< 9	Negative
0.9-1.1	9-11	Equivocal
>1.1	>11	Positive

Dengue IgG capture ELISA

Principle of the test: Serum antibodies of the IgG class, when present, combine with anti-human IgG antibodies coated on the polystyrene surface of the microwell test strips (assay plate). An equal volume of horseradish peroxidase (HRP) conjugated monoclonal antibody (MAb) is added to the diluted antigen, allowing the formation of antigen-MAb complexes. Residual serum is removed from the assay plate by washing, and complexed antigen-MAb complexes added then bind to the serum dengue-specific IgG antibodies. After incubation a colourless substrate

added to the washed wells and was followed by tetramethylbenzidine/hydrogen peroxide (TMB Chromogen) addition. The substrate is hydrolysed by HRP if present, and the chromogen turns blue. After stopping the reaction with acid, the TMB turns yellow, Colour development is indicative of the presence of anti-dengue IgG antibodies in the test sample.

Preprocedure preparation:

All the kit reagents were brought to room temperature before the commencement of the procedure.

Procedure:

Antigen diluted 1/250 using antigen diluent i.e. 10µl antigen mixed with 2.5ml of antigen diluent .About 0.5ml of diluted antigen is required per strip.

Equal volume of diluted antigen mixed with monoclonal antibody tracer solution. This is incubated at room temperature for 1 hour.

1. Dispensed 100 µl of diluted sample, positive control, negative control and calibrators in triplicate to the respective wells
2. The plate was covered and Incubated at 37 °c for 1 hour.
3. Plate was washed 6 times with diluted wash buffer at the end of incubation

4. Added 100 μ l of Ag –MAb tracer solution to all the wells at the end of incubation
5. Plate was covered and Incubated at 37⁰c for 1 hour.
6. Washed 6 times with wash buffer at the end of incubation.
7. Added 100 μ l of TMB was to all the wells.
8. Incubated at dark for 10 minutes.
9. Added 100 μ l of stop solution to all the wells
10. Absorbance value of each well measured at 450 nm wavelength with 600-650 nm reference filter spectrophotometrically.

Calculation:

1. The average absorbance of the triplicate of the calibrator is multiplied by the calibration factor. This is the cut-off value.
2. Index value is calculated by dividing the sample absorbance by the cut- off value.

Test validity:

Cutoff value= 1.5x neg.absorbance

Calibrator mean >negative absorbance

Positive control/Cutoff value= 1.1-6.0

Negative control<.350

Calibration factor=1

Index value= Sample absorbance / cutoff value

Panbio units= index value x 10

Interpretation of results:

INDEX	PANBIOUNITS	RESULT
<1.8	<18	Negative
1.8-2.2	18-22	Equivocal
>2.2	>22	Positive

The haematological and biochemical profile of dengue positives were estimated in the respective Departments and results were collected.

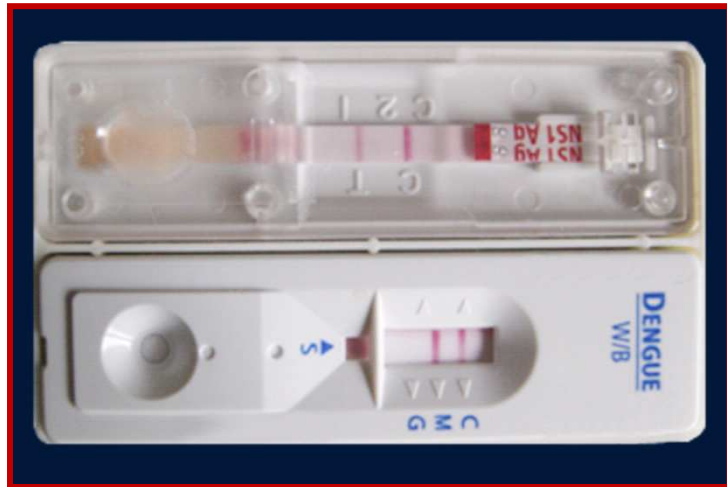
Dengue Duo Combo Card Kit



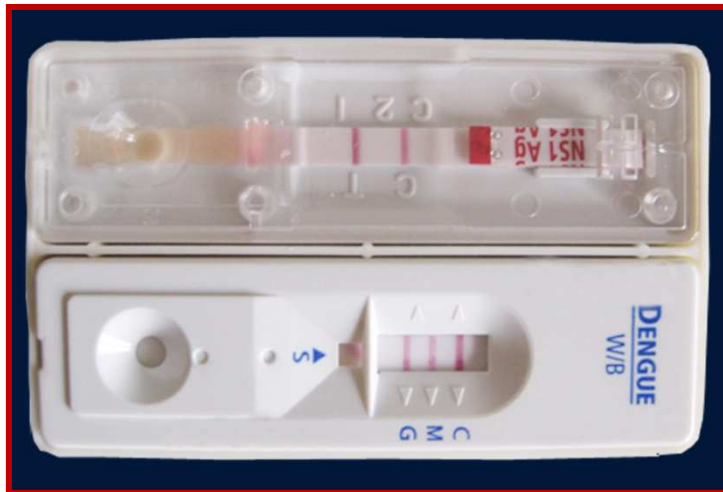
NS1 Positive



NS1 with IgM Positive



NS1 with IgM and IgG Positive



NS1 with IgG



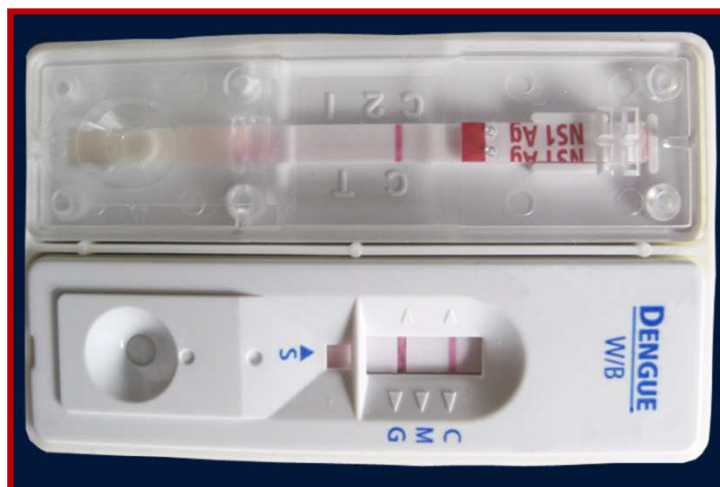
NS1 Negative with IgM and IgG Positive



IgM antibody positive



IgG Antibody Positive



NS1 Antigen ELISA Kit



IgM ELISA Kit



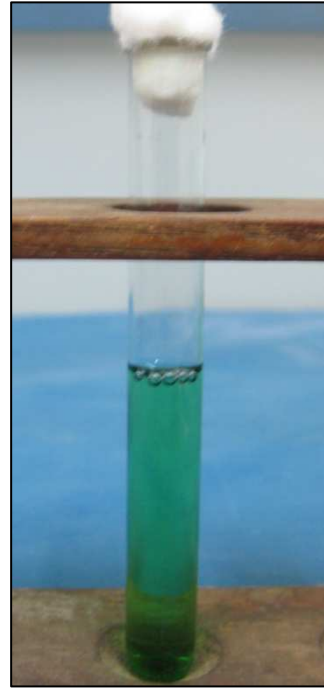
IgG ELISA Kit



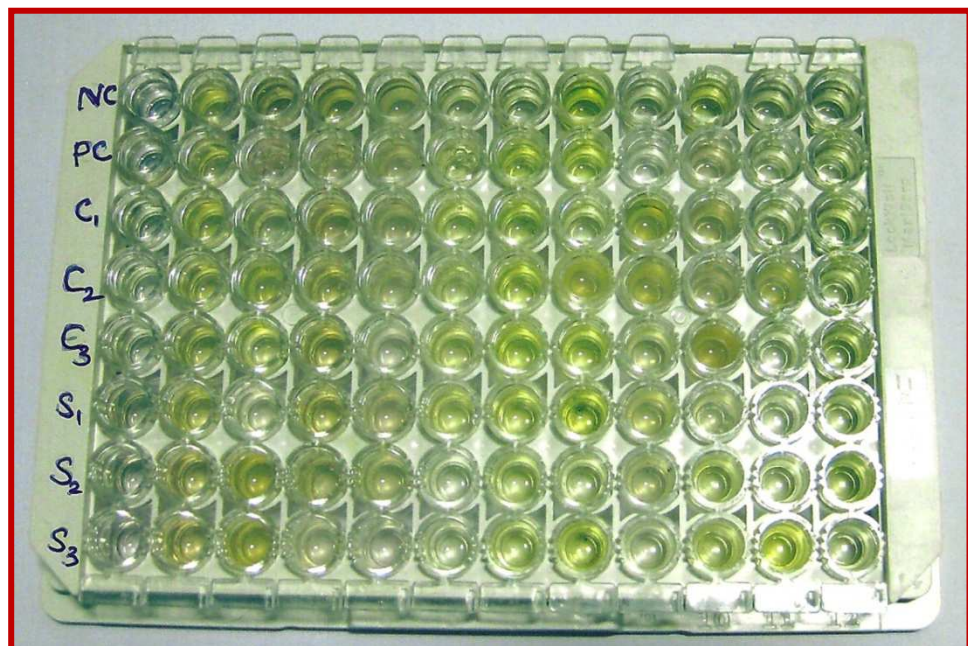
ELISA Reader



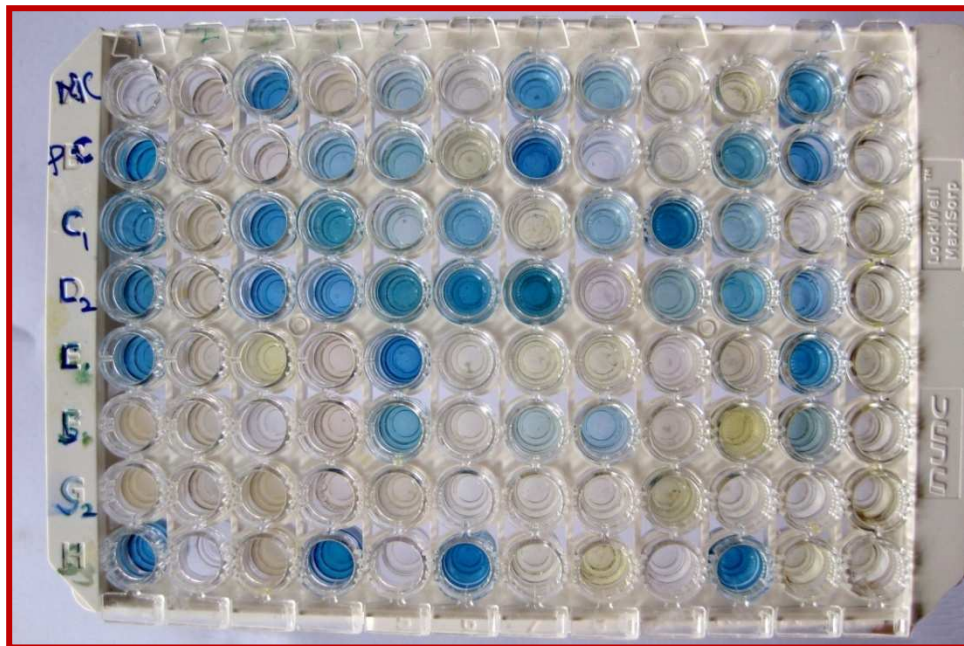
Antigen Antibody Mixture of IgM & IgG



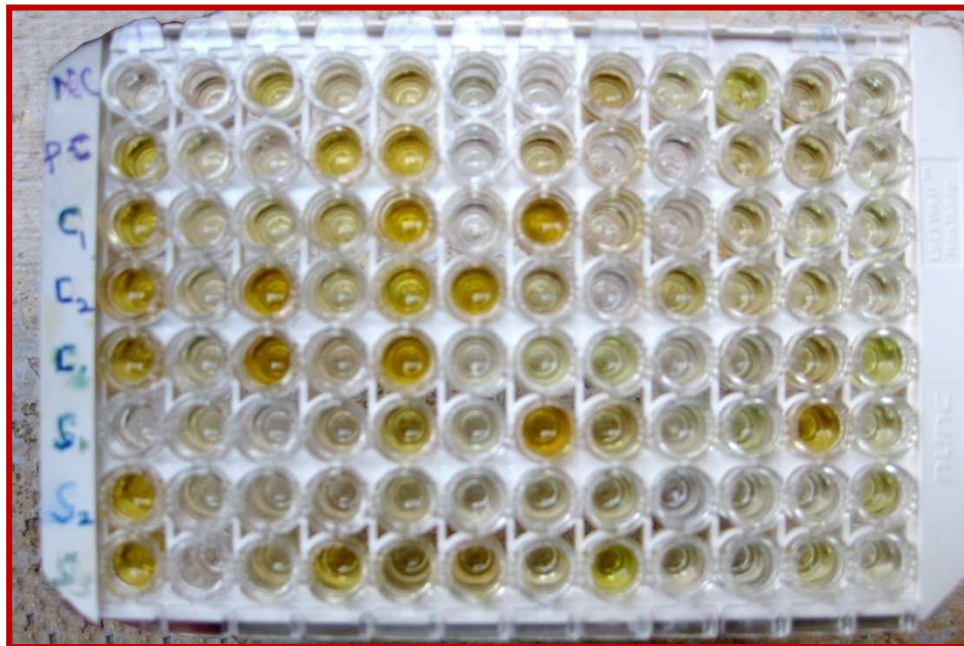
MicrotitrePlate with Samples



Microtitre Plate with Samples after substrate Addition



Samples after addition of Stop Solution



RESULTS

During the study period of one year Blood was collected from 350 clinically suspected dengue cases. The samples were subjected to Dengue serology for both NS1 antigen and IgM, IgG antibody detection by ELISA and ICT. The 107 seropositive samples by ELISA were further analysed for demographic, clinical and laboratory data including haematological and biochemical profile. The Statistical analysis of results of this study was done using SPSS version 17. p value obtained from Pearson chi-square test.

In this study the seropositivity of dengue was 30 % (Ref Table 1).

The age of the seropositives ranged from 0-72 years and the commonest age group affected in this study was 0-10 years 45 (42%) followed by 11-20 years 27 (25.2%), 21-30 years 16 (14.9%), 31-40 years 12 (11.2%). The incidence was less in the age group of 41-50 years and 51-60 years each constituting about 2 (1.8%) and >60 years 3 (2.8%). (Ref Table 2)

The male: female ratio was 1.6:1 in this study with males 67 (62.3%) and females 40 (37.7%) (Ref Table 2).

The incidence of dengue cases in this study month wise were September 2 (16.6%), October 0 (0%), November 5 (21.7%), December 12

(25.5%), January 13 (31.7%), February 2 (13.3%), March 1 (4.3%), April 4 (23.5%), May 13 (26.5%), June 41 (50.6%) and July 13 (52%) and August 1 (12.5%). (Ref Table 3).

The common clinical symptom was fever 107 (100%), followed by headache 65(60.7%), Myalgia 41(38.3%), Arthralgia 32 (29.9%), Abdominal pain 30 (28.03%), vomiting 26 (24.2%) , Oliguria and Rash was observed in 5 cases(4.6%).(Ref Table 4)

Melena 11(10.2%) was the commonest bleeding manifestation followed by bleeding gums 5 (4.6%), Hematemesis 4 (3.7%) and Epistaxis 3 (2.8%) (Ref Table 4)

The positive cases were classified as Dengue fever 52(48.5%), Dengue haemorrhagic fever 53(49.5%) and Dengue shock syndrome 2(2%) according to WHO guidelines. (Ref Table 5).

In this study thrombocytopenia was associated with majority of Dengue positive cases .The common range was 51,000-1lakh 44 (41.1%) followed by 21000-40,000 30(28.03%), <20,000 13(12.14%), 41,000-50,000 10(9.34%) and >1lakh 10(9.34%). Among thrombocytopenia <1lakh 43(82.6%)

were associated with dengue fever, 52 (98%) with Dengue haemorrhagic fever and 2 (100%) with Dengue shock syndrome. (Ref Table 6 & 9).

In this study leucopenia (count <4000) was observed in 79(73.8%) dengue positive cases. Out of 52 Dengue fever cases 37 had leucopenia(71.1%), out of 53 DHF 40 had leucopenia(75.4%) and out of 2 DSS both had leucopenia(100%). Eight cases (7.5%) had WBC count of 4000-5000, 7 were in 5100-6000(6.5%) range, 6 were in 6100-7000 range and 7 cases had count >7000(6.5%). (Ref Table 7&9).

Elevated Haematocrit > 45% was observed in 33(30.8%) cases. Among them 5(9.6%) were associated with Dengue fever and 26(49.05%) with Dengue haemorrhagic fever and 2(100%) with Dengue shock syndrome. HCT level in other cases were 35-44 in 30(28.03%) cases, 25-34 in 34(31.7%) and <25 in 10 cases (9.3%). (Ref Table 8&9)

Liver parameters like ALT and AST were raised in 24(22.4%) and 28(26.1%) cases respectively. Among ALT elevated cases 8 (15.3%) were associated with Dengue fever, 14(26.4%) were associated with Dengue haemorrhagic fever and 2(100%) were associated with Dengue shock syndrome. Among AST elevated cases 10 (19.2%) were associated with

Dengue fever, 16(30.1%) were associated with Dengue haemorrhagic fever and 2(100%) were associated with Dengue shock syndrome. (Ref Table 10)

In Ultrasonogram (USG) Gallbladder thickening 31(28.9%) was observed in majority of Dengue positive cases followed by pleural effusion 16 (14.9%), ascites 9 (8.4%) and hepatomegaly 7(6.5%). Among GB thickening 5 (9.6%) were associated with Dengue fever, 24 (45.2%) with Dengue haemorrhagic fever, 2(100%) with Dengue shock syndrome .Pleural effusion of 14(26.4%) were associated with Dengue haemorrhagic fever and 2(100%) were associated with Dengue shock syndrome and nil case in Dengue fever. Ascites of 7(13.2%) were associated with Dengue haemorrhagic fever, 2(100%) with Dengue shock syndrome and nil case in Dengue fever. Hepatomegaly was associated with dengue fever 5 (9.6%) and Dengue haemorrhagic fever 2(3.7%) and nil association with Dengue shock syndrome.USG was normal in 30(28.03%) cases which was 25 (48%) in Dengue fever and 5(9.4%) in Dengue haemorrhagic fever.USG was not available for 17 cases of Dengue fever and 1case in Dengue haemorrhagic fever.(Ref Table 11)

Seropositives were categorised as Primary 48(44.9%) and secondary dengue 59(55.1%)with ratio0.8:1 based on ELISA .The serological markers

included for primary dengue were NS1 alone positive ,NS1 with IgM positive, IgM alone positive and IgM&IgG positive with ratio >1 ⁵⁰. The serological markers and their combination included for secondary dengue were IgG positive with thrombocytopenia, NS1 & IgG positive, NS1 ,IgM & IgG positive and IgM & IgG positive with ratio <1 ⁵⁰.(Ref Table 12)

Based on duration of fever ,primary dengue cases with above mentioned markers were split into 1-5 days(37),6-10 days(10) and >10 days(1).

In primary Dengue(PD) NS1 alone showed positivity of 27(56.25%), NS1 & IgM showed positivity of 8(16.6%) , IgM alone showed positivity of 9(18.75%), IgM & IgG +ve with ratio >1 showed positivity of 4(8.33%).

In 1-5 days period NS1 alone showed positivity of 27(72.9%) followed by NS1 & IgM positivity of 8(21.6%) and IgM, alone positive of 2(5.4%). In 6-10 days duration IgM alone showed positivity of 70% followed by IgM & IgG positive with ratio >1 positivity of 3(30%). NS1 and NS1 with IgM showed nil positivity. In >10 days IgM & IgG positive with ratio >1 alone had positivity of 100% ,others were nil during this period. With

p value<0.001 it was found to be statistically significant. (Ref Table 13)

Similarly in secondary dengue(SD) IgG positive with thrombocytopenia showed positivity of 6(10.1%) , NS1 & IgG of 1(1.6%) , NS1,IgM &IgG of 14(23.7%), IgM &IgG positive with ratio <1 of 38(64.4%) .

In 1-5 days of secondary dengue NS1,IgM&IgG 11(47.8%) showed higher positivity followed by IgM & IgG positive with ratio <1 6(26.08%), IgG positive with thrombocytopenia were 5(21.7%),NS1&IgG positive 1(4.3%).

In 6-10 days IgM &IgG positive with ratio <1 showed positivity of 22(84.6%) followed by NS1,IgM,IgG positivity of 3(11.5%)and IgG positive with thrombocytopenia1(3.8%). In > 10 days duration IgM &IgG positive with ratio<1 showed higher positivity of 10(100%).Others showed nil positivity. With p value<0.001 it was found to be statistically significant.(Ref Table 14)

Case detection by ICT and ELISA showed 26.2% positivity for ICT and 30.5% positivity for ELISA. (Ref Table 15)

Detection of serological markers like NS1, IgM and IgG by ICT and ELISA showed positivity of 36(10.2%), 62(17.7%), 54(15.4%) by ICT and 50(14.2%), 73(20.8%), 65(18.2%) respectively by ELISA (Ref Table 15a).

Using ELISA as Reference assay, Sensitivity, Specificity, NPV and PPV of the ICT were calculated for NS1 which showed 68%, 99.3%, 94.4%, and 85.7% respectively. With p value of <0.001 it was found to be statistically significant.(Ref Table 16)

Similarly for IgM the ICT showed sensitivity, specificity, PPV, NPV of 80.8%, 98.9%, 95.1%, and 95.1% respectively. With p value <0.05 it was found to be statistically significant.(Ref Table 17).

Sensitivity, Specificity, PPV, and NPV of IgG in ICT was 81.3%, 98.9%, 94.1%, 96.32% respectively. With p value <0.001 it was found to be statistically significant. (Ref Table 18)

Tables

Table 1

Seropositivity of Dengue

Total No. of Suspected fever Cases	Total No. of positive cases	Percentage
350	107	30.5%

Table 2

Age and Sex Wise Distribution of Dengue positive cases

S.No.	Age Group	Male	Female	Total
1.	0-10 Yrs	26(24.2%)	19(17.7%)	45(42%)
2.	11-20 Yrs	18(16.8%)	09(8.4%)	27(25.2%)
3.	21-30 Yrs	10(9.3%)	06(5.6%)	16(14.9%)
4.	31-40 Yrs	10(9.3%)	02(2.0%)	12(11.2%)
5.	41-50 Yrs	01(0.9%)	01(1%)	02(1.8%)
6.	51-60 Yrs	-	02 (2.0%)	02(1.8%)
7.	>60 Yrs	02(1.8%)	01(1.0%)	03(2.8%)
Total		67(62.3%)	40(37.7%)	107(100%)

Male: Female ratio 1.6:1

Table 3

Seasonal Distribution of Dengue positive Cases

Month	Total no of cases	No of Positives	Percentage
2011-2012	350	107	%
September	12	2	16.6
October	9	0	0
November	23	5	21.7
December	47	12	25.5
January	41	13	31.7
February	15	2	13.3
March	23	1	4.3
April	17	4	23.5
May	49	13	26.5
June	81	41	50.6
July	25	13	52
August	8	1	12.5

chi square test =30.7, p value<.001(significant)

Table 4

Clinical Presentation of Dengue positive cases

Symptom	No. of Patients	Percentage
Fever	107	100%
Headache	65	60.7%
Myalgia	41	38.3%
Arthralgia	32	29.9%
Abdominal Pain	30	28.03%
Vomiting	26	24.2%
Melena	11	10.2%
Oliguria	5	4.6%
Bleeding gums	5	4.6%
Rash	5	4.6%
Hemetemesis	4	3.7%
Epistaxis	3	2.8%

Table 5

Clinical Classification based on WHO Guidelines⁵¹

Category	No. of Positive Cases	Percentage
Dengue Fever	52	48.5%
Dengue Hemorrhagic Fever	53	49.5%
Dengue Shock Syndrome	2	2%
Total	107	100%

chi -square test =47.68, p<0.001(significant)

Table 6

Platelet Count in Dengue positive cases (n = 107)

Platelet Count	Total cases	Percentage %
<20,000	13	12.14
21,000 – 40,000	30	28.03
41,000 – 50,000	10	9.34
51,000 – 1 lakh	44	41.12
>1 Lakhs	10	9.34
Total	107	100

chi-square test=42.766, pvalue<0 .001(significant)

Table 7

WBC Count of Dengue positive cases (n = 107)

WBC Count	Total cases	Percentage %
< 4000	79	73.8
4000 – 5000	8	7.5
5100 – 6000	7	6.5
6100 – 7000	6	5.6
>7000	7	6.5
Total	107	100

chi-square test=42.7, p value<0.001(significant)

Table 8

Level of HCT in Dengue positive cases (n = 107)

HCT	Total cases	Percentage %
>45	33	30.8
35-44	30	28.03
25-34	34	31.7
<25	10	9.3
Total	107	100

chi square test= 42.7, p value <0.001 (significant)

Table 9

Association of platelet, Haematocrit and WBC with Dengue positives

Category	Platelet count < 1 lakh(97)	Haematocrit >45(33)	WBC count <4000(79)
Dengue fever(52)	43(82.6%)	5(9.6%)	37(71.1%)
Dengue hamorrhagic fever(53)	52(98.1%)	26(49.05%)	40(75.4%)
Dengue shock syndrome(2)	2(100%)	2(100%)	2(100%)
Total	97	33	79

Table 10

Association of ALT and AST in Dengue positive cases

Category	ALT>40(24)	AST>40(28)
Dengue fever(52)	8(15.3%)	10(19.2%)
Dengue haemorrhagic fever(53)	14(26.4%)	16(30.1%)
Dengue shock syndrome(2)	2(100%)	2(100%)
Total	24(22.4%)	28(26.1%)

chi-square test=12.1, pvalue <0.05(significant)

Table 11

USG Findings in Dengue positive cases.

Category	Normal %	GB thickening %	Pleural effusion %	Ascites %	Hepatomegaly %
Dengue fever(52)	25(48)	5(9.6)	0	0	5(9.6)
Dengue haemorrhagic fever (53)	5(9.4)	24(45.2)	14(26.4)	7(13.2)	2(3.7)
Dengue shock syndrome(2)	0	2(100)	2(100)	2(100)	0
Total	30(28.03)	31(28.9)	16(14.9)	9(8.4)	7(6.5)

Table 12

Serological Categorisation of Dengue positives based on ELISA

Category	No. of Positive Cases	Percentage	Ratio
Primary Dengue	48	44.9%	0.8:1
Secondary Dengue	59	55.1%	

Table 13

Primary dengue based on duration of fever & serological markers

Fever duration	NS1 positive	NS1&IgM Positive	IgM alone Positive	IgM &IgG+ve with Ratio >1
1-5 Days (37)	27 (72.9%)	8 (21.6%)	2 (5.4%)	0 (0%)
6-10 Days (10)	0 (0.0%)	0 (0.0%)	7 (70%)	3 (30%)
> 10 days (1)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (100%)
Total (48)	27 (56.25%)	8 (16.6%)	9 (18.75%)	4 (8.33%)

chi-square test=47.07, p value<0.001(significant)

Table 14

Secondary Dengue based on duration of fever and serological markers

Fever duration	IgG+thrombocytopenia	NS1 &IgG Positive	NS1,IgM&IgG Positive	IgM&IgG+ve with Ratio <1
1-5 Days (23)	5 (21.7%)	1 (4.3%)	11 (47.8%)	6 (26.08%)
6 – 10 Days (26)	1 (3.8%)	0 (0.0%)	3 (11.5%)	22 (84.6%)
> 10 Days (10)	0 (0.0%)	0 (0.0%)	0 (0.0%)	10 (100%)
Total (59)	6 (10.1%)	1 (1.6%)	14 (23.7%)	38 (64.4%)

chi-square test= 25.12, P value<0.001(significant)

Table 15

Case detection by ICT and ELISA

Test	No. of Suspected Cases	No. of Positive Cases	Percentage
ICT	350	92	26.2%
ELISA	350	107	30.5%

Table 15(a)

Detection of NS1, IgM, IgG by ICT and ELISA

Test	No of suspected cases	ICT	ELISA
NS1	350	36(10.2%)	50(14.2%)
IgM	350	62(17.7%)	73(20.8%)
IgG	350	54(15.4%)	65(18.2%)

Table 16

Cross tabulation of ICT and ELISA for NS1

ICT	ELISA		Total
	Positive	Negative	
Positive	34	2	36
Negative	16	298	314
Total	50	300	350

Sensitivity 68%, Specificity 99.3%, PPV 94.4%, NPV 85.7%

chi-square test =46.36, p value<0.001(significant)

Table 17

Cross tabulation of ICT and ELISA for IgM

ICT	ELISA		Total
	Positive	Negative	
Positive	59	3	62
Negative	14	274	288
Total	73	277	350

Sensitivity 80.8%, Specificity 98.9%, PPV 95.1%, NPV 95.1%
chi-square test =5.1, p value < 0.05 (significant)

Table 18

Cross tabulation of ICT and ELISA for IgG

ICT	ELISA		Total
	Positive	Negative	
Positive	51	3	54
Negative	14	282	296
Total	65	285	350

Sensitivity 81.3%, Specificity 98.9%, PPV 94.1%, NPV 96.32%
chi-square test =41.3, p value < 0.001 (significant)

Chart 1
Seropositivity of Dengue

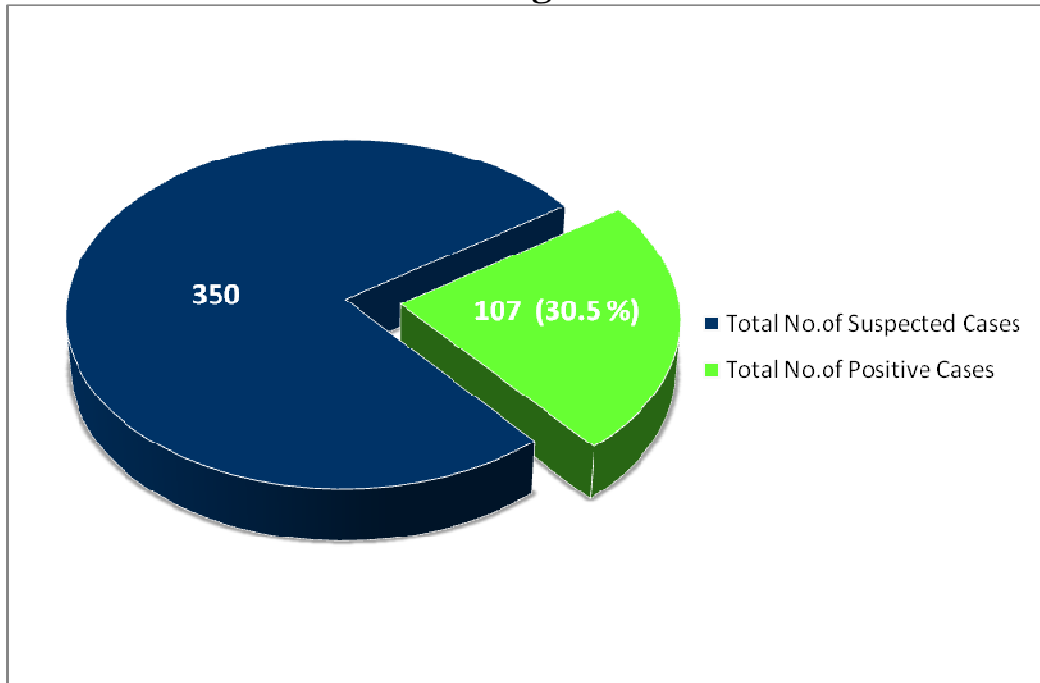


Chart 2
Age & Sex wise Distribution of Dengue Positive Cases

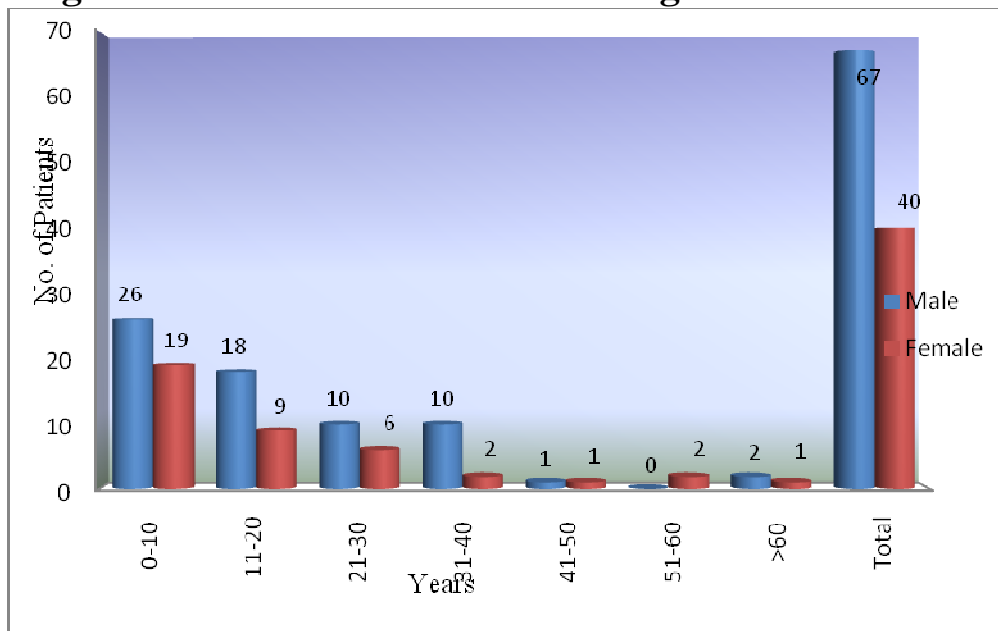


Chart 3
Seasonal Distribution of Dengue Positive Cases
from 2011 to 2012

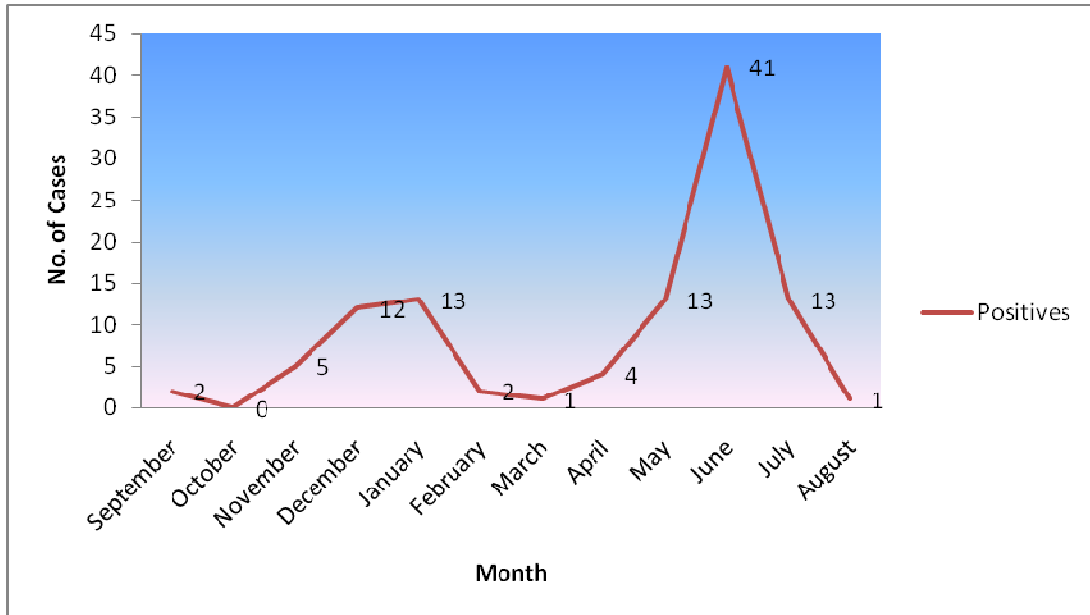


Chart 4
Clinical presentation of Dengue Positive Cases

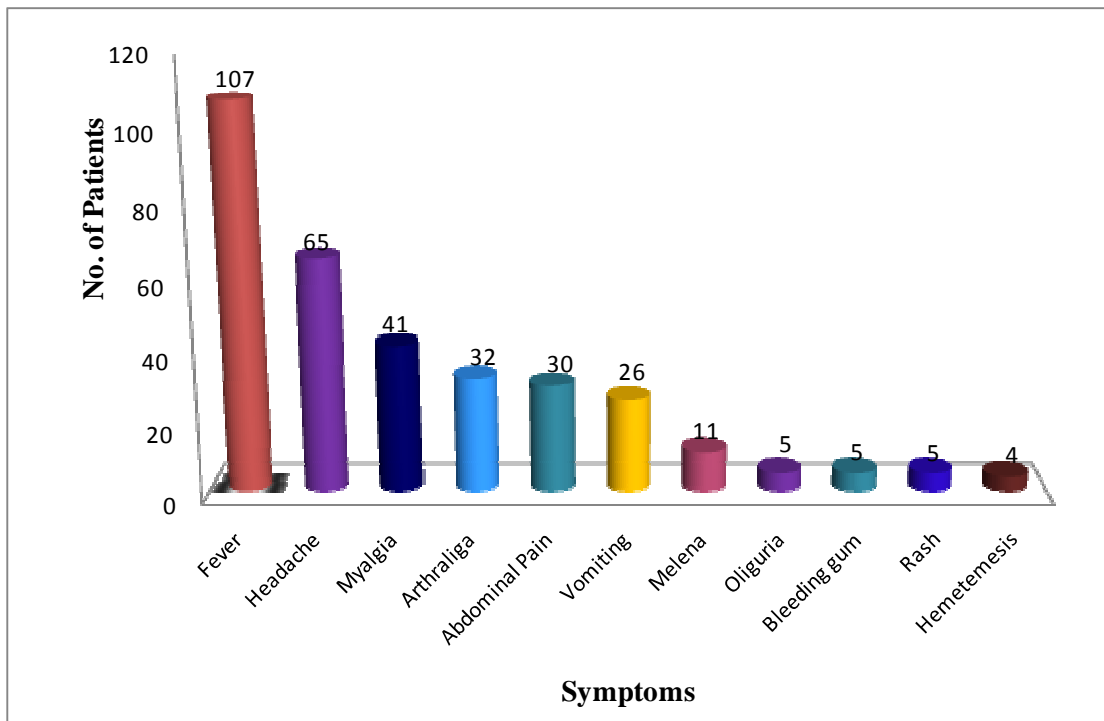


Chart 5
Clinical Classification based on WHO Guidelines

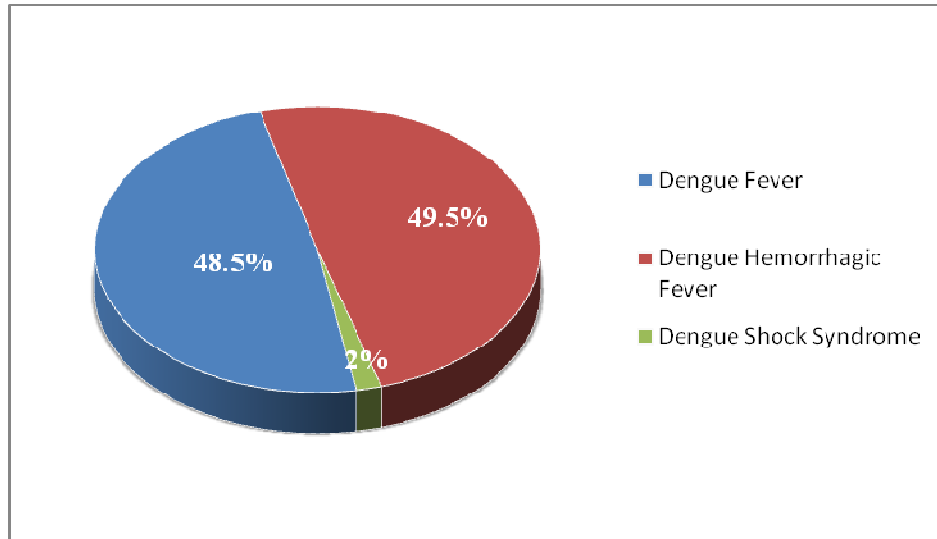


Chart 6
Association of Platelet, WBC, Hematocrit with DF, DHF and DSS

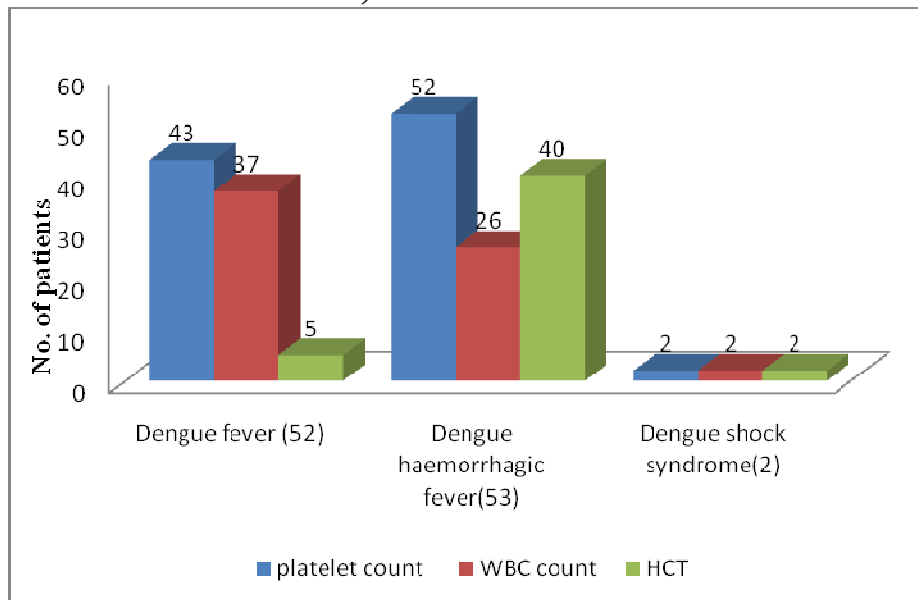


Chart 7
Serological Categorisation of Dengue Positive Cases

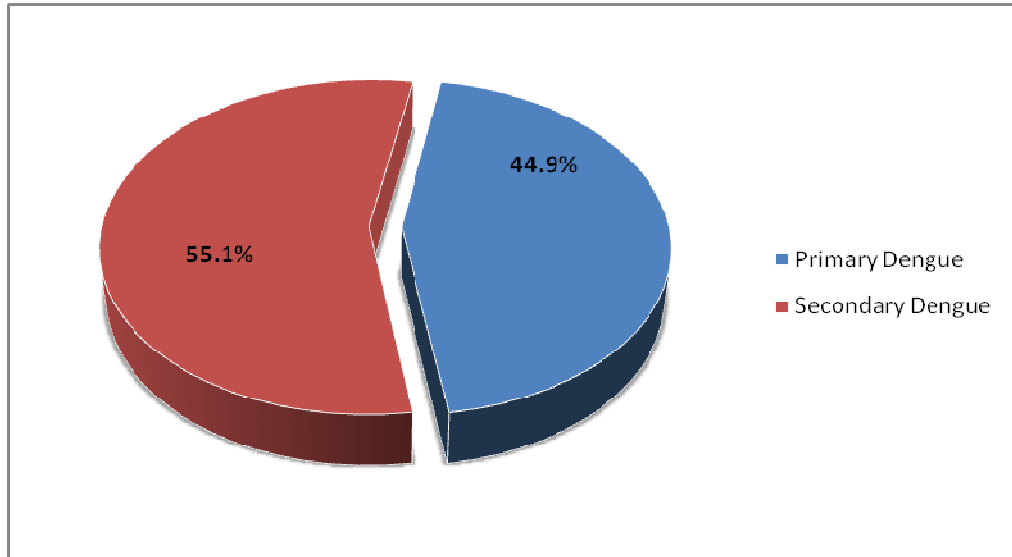


Chart 8
Primary dengue based on duration of fever and serological markers

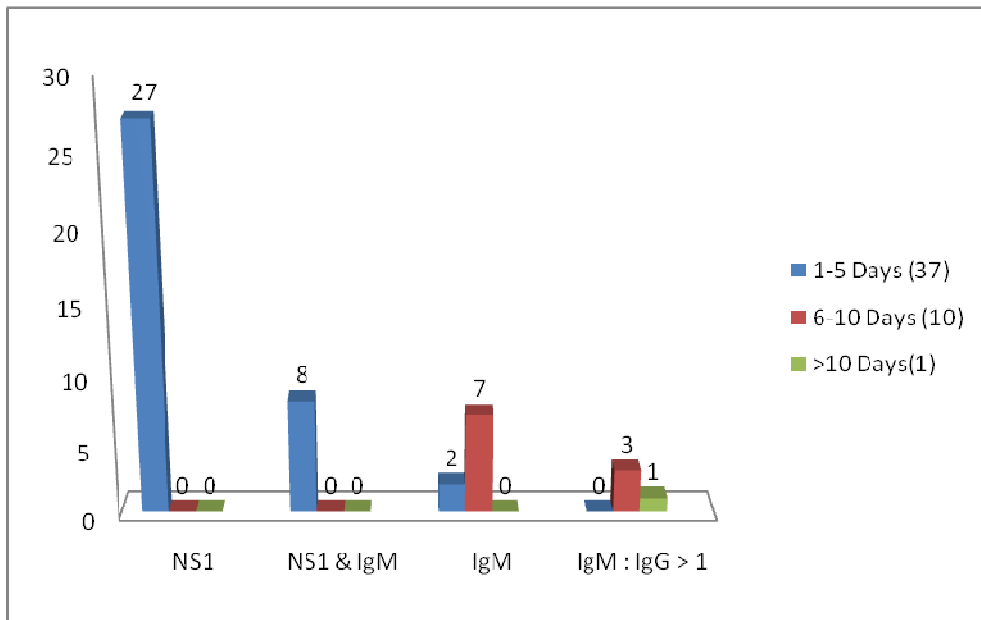


Chart 9
Secondary Dengue based on duration of fever and serological markers.

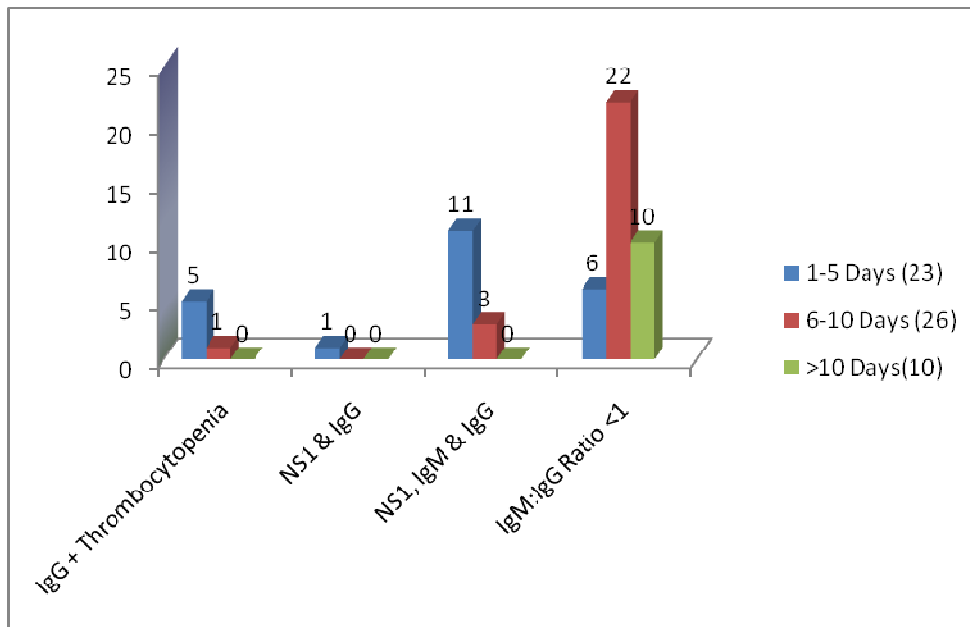


Table 10
Detection of NS1, IgM & IgG by ICT and ELISA

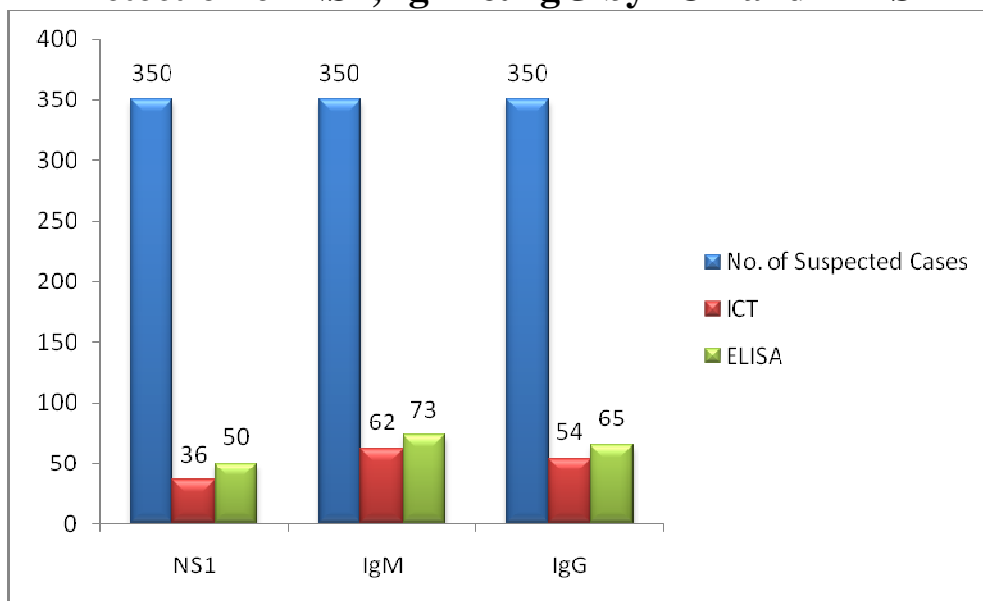
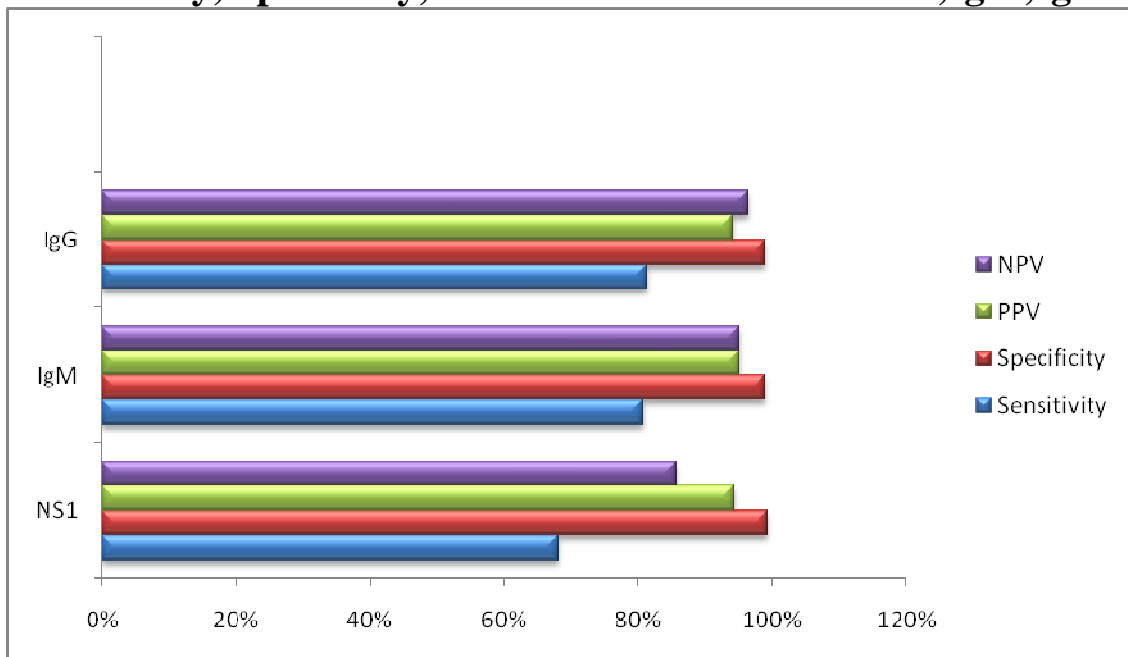


Chart 11
Sensitivity, Specificity, PPV & NPV of ICT for NS1,IgM,IgG



DISCUSSION

In order to provide timely information for the management of patients with acute dengue and effectively control dengue outbreaks, it is important to establish an accurate confirmation of acute dengue infection during the first few days of clinical symptoms.

For a long time, detection of dengue specific IgM/IgG has been the main stay of diagnosis of Dengue infection. The Dengue specific IgM antibodies begin to appear only around fifth day of primary infection. The New parameter called NS1 now available can be detected from the first day of illness. Dengue NS1 is detected in the blood circulation as early as viral RNA detected in PCR.

In this study the seropositivity of dengue infection was 30% .In a study by **Halstead et al**⁵² 1969, Bangkok 29% of febrile illness was associated with dengue virus infection similar to this study.

In a study by **Chakravarti A et al**⁵³ 2011, New Delhi conducted on 145 samples noted seropositivity of 60.7% which is higher to this study.

In this study 62.3% of the affected populations were males and 37.7% were females. The following studies were found to coincide with this study **Aisha**

sajid, Asim akram, Mubashir Ahmed⁵⁴ 2012, **Syed Irfan ahmed et al**⁵⁵ 2010 Fasalabad and **Kurukumbi et al**²¹ 2001.

In contrast to this study females were affected more than males in a study by **Murray smith and Skelly C**⁵⁶ Queensland. But **Susan shepherd et al**⁵⁷ in a study conducted at Pennisylvania showed that dengue infection can occur irrespective of sex.

In this study Male: female ratio was 1.6:1 and this is due to males being engaged in outdoor activities and due to their dressing culture when compared to females which increases the exposure to mosquito bites. The studies by Eng **Eong Ooi**⁵⁸ 2001 Singapore, **Shrivastava et al**⁵⁹ 2011 and **Rajoo Singh Chhina et al**⁶⁰ 2008 Punjab had similar M: F ratio.

In this study the commonest age group affected was 0-10 years (42.05%) in both sexes. Increased incidence of infection in 0-10 years in this study is due to increased mosquito bite exposure in the school premises during the day time. The study by **Shah GS et al**⁶¹ Kathmandu revealed that the commonest age group affected in their study was from 8months to 14 years and **Malavige et al**⁴⁵ 2004 Srilanka study showed age range from 1 month to 12 years which is similar to this study.

In a study by **Anita chakravarti et al**⁶² conducted at New Delhi the age group commonly affected was 26-30 years which is in contrast to this study.

In this study incidence of dengue cases were more during the months of November & December 2011 and January, May, June, July of 2012. Increase in the incidence of Dengue cases occur usually during rainfall⁶³. Tamilnadu receives rainfall from October to November followed by post monsoon period of December & January. The cause for the incidence of cases during the above months like May, June and July in this study is that the eggs can survive even in stored waters in the absence of rainfall. According to **NVBCDP**²⁵ 2007 every year there is an upsurge in the cases of Dengue /DHF from July to November. **Ekta gupta et al**⁶⁴ 2006 in their 3 year study had shown an increase in incidence during Post monsoon period (September – November).

A study by **Louise Kelly –hope Mandeleine C.Thomson**⁶⁵ 2008 in their Study revealed that a climatic change has a role in the transmission of Dengue. **Umesh Isalkar**⁶⁶, TNN 2009 said that weather changes cause atypical viral fever.

In this study the commonest symptom was fever 100% followed by headache 60.7%, Myalgia 38.3%, arthralgia 29.9%, abdominal pain 28.3%

,vomiting 24.2% ,oliguria &,rash 4.6% . **Saadiah sulaiman et al**⁶⁷1999 in their study had fever of 100% followed by headache (80.9%) arthralgia (74%), myalgia (70%), rash (4.6%) and vomiting (62.7%) similar to this study. **Jonathen et al**⁶⁸ 2010 in their study had fever (72%) as most common symptom followed by epistaxis, abdominal pain and rashes.

Rahman et al⁶⁹ 2002 in their study had headache (90%) as predominant symptom followed by myalgia / arthralgia (85%) and vomiting (64%).

In this study Dengue positive cases were classified according to WHO guidelines. It showed 48.5% Dengue fever, 49.5% Dengue haemorrhagic fever and 2% Dengue shock syndrome. In this study DHF is found to be high because of increased incidence of infection in children. Because of the increased surface area of the blood vessel per unit volume of skeletal muscle and the immature blood vessel leading to increased vascular permeability, DHF is common in children. Even though Dengue haemorrhagic fever was high in this study and is said to be a complication of secondary dengue, DSS was comparatively less because of the eminent care and management given to the diagnosed cases in the respective wards.

In a study by **S Kalyanarooj et al**⁷⁰ 1997 Thailand, conducted on 60 seropositives showed 53% (32) Dengue fever, 47% (28) Dengue

haemorrhagic fever and Dengue shock syndrome. According to **David W. Vaughn and Sharone Green**⁷¹ fatality rate is decreased in DHF /DSS cases those who are hospitalised and treated promptly.

In this study thrombocytopenia was observed in 90% of the positive cases the common range was between 51,000-1 lakh followed by 20,000- 40,000. Thrombocytopenia is due to decreased platelet production in Dengue fever and increased peripheral destruction in DHF. **RD Kulkarni et al** ⁶2011 Karnataka, in their study had thrombocytopenia associated with majority of dengue positive cases than dengue negative cases similar to this study. In a study by **Chandrakanta et al**⁷² **2008** revealed 60% of thrombocytopenia < 1lakh.

Karande et al⁷³ 2005 revealed that arthralgia and thrombocytopenia if present in a child increases the chance of the child to be Dengue positive. But a study by **Col Banerjee et al**⁷⁴ revealed that thrombocytopenia may not always be a feature of Dengue.

In this study leucopenia was observed in 79/107(73.8%) of dengue positive cases. Out of 79, 37 were associated with dengue fever, 40 were associated with Dengue haemorrhagic fever, 2 were associated with Dengue shock syndrome. The association of leucopenia with Dengue fever, DHF and DSS is

71.1%,75.4% and 100% respectively(Table 9). Neutrophil adhesion to dengue infected endothelial cell is one of the causes for leucopenia.

Kalyanarooj et al⁷⁰ 1997 has stated that leucopenia in Dengue is due to bone marrow suppression.In a study by **Somachai insiripong MD**⁷⁵ 2010 revealed that leucopenia in DHF is multifactorial .

In this study elevated Heamatocrit (>45%) was observed in 33 patients, 5 were associated with dengue fever, 26 were associated with Dengue haemorrhagic fever and 2 were associated with Dengue shock syndrome. The association of elevated HCT with DF, DHF and DSS are 9.6%, 49.05% and 100% respectively. Rise in haematocrit is due to increased vascular permeability and plasma leakage in DHF /DSS. **Malavige et al**⁷⁶2007 in their study have considered hemoconcentration when the packed cell volume was >45 in children.

In this study liver enzymes like AST (Aspartate aminotransferase)) and ALT (Alanine aminotransferase) were elevated in 28 and 24 patients respectively. Among ALT and AST rise, >40units majority were associated with DHF and DSS. **Rajoo Singh Chhina et al**⁶⁰, Punjab in their study had significant rise of AST (97%) and ALT (93%) in DHF/DSS as compared to

DF. They also concluded that raised AST can be an early indicator of dengue infection.

In secondary dengue causing liver damage AST will be elevated more than ALT which is due to the release of AST from damaged monocytes of dengue infection and due to release of AST from skeletal muscles due to myalgia.

In this study, the Ultrasound findings showed more of Gall bladder thickening followed by pleural effusion, ascites and hepatomegaly . Gall bladder thickening is due to increased vascular permeability where the fluid tends to collect in between the layers increasing the thickness of the Gall bladder which occurs in Dengue haemorrhagic fever and Dengue shock syndrome. Similarly the fluid collects in pleural and peritoneal cavity.

In the study conducted by **Bala subramanian et al**⁷⁷2006 ultrasonography was found to be superior to radiography in detecting pleural effusion. In the study by **Kumar et al**⁷⁸ 2010 Karnataka, pleural effusion was noted in 4.29 % (20) of 466 patients which is less than this study. In this study by **Zulkarnain**⁷⁹ 2004 revealed 29.8% of Gall bladder edema which is higher than this study.

In this study the incidence of Primary dengue was 48(44.8%) and Secondary Dengue was 59(55.1%) in the proportion of **0.8:1**. In a study by **Jonathan G et al**⁶⁸ 2010 secondary dengue (81%) was more than primary dengue which is similar to this study. In a study by **Neeraja M et al**²², Hyderabad showed 72.5% of secondary dengue conducted on 211patients which is similar to this study.

In 48 samples of primary dengue NS1 showed higher positivity (56.25%) followed by IgM alone positivity of 18.75% , NS1 &IgM positivity of 16.6% , and IgM & IgG positive with ratio >1of 8.33%.During the primary infection ,NS1 gets secreted out from virus infected cells which indicates underlying viremia and pathogenesis of infection. As there are low antibody titres in primary dengue, the NS1 protein can be estimated more in serum during the acute period. So NS1 detection can be used as a stand alone test for Primary dengue in cases when IgM is not detectable and where PCR is not available.

During the acute period of 1-5 days in primary dengue , NS1 detection (72.9%) had higher positivity followed by NS1 & IgM(21.6%) and IgM alone positive of 2(5.4%). As IgM antibodies tends to appear after 4-5 days of infection and as many patients in this study presented within 5 days, the

sensitivity of NS1 & IgM, IgM alone positive in primary dengue were less when compared to NS1 positivity alone. Hence when IgM ELISA is negative in the acute period, NS1 ELISA should be done for diagnosis which significantly improves the detection rate without the requirement of paired serum to demonstrate the rise in titre of antibodies. In a study by **Chakravarti A, Kumar A, Malik S**⁵³ 2011 New Delhi conducted on 145 acute serum samples NS1 ELISA showed a positivity rate of 65.9% and MAC-ELISA showed 60.2% positivity in the acute period. **Veasna duong et al**⁹ study also showed NS1 positivity to be higher in first 3 days. **Minipritam singh et al**⁸⁰ and **Chua KB et al**⁸¹ 2011 studies revealed that NS1 antigen capture ELISA was more sensitive than RT-PCR and viral isolation.

During the early convalescent period of 6-10 days in primary dengue, IgM showed higher positivity (70%) than other serological markers. This is due to the appearance of IgM antibodies after 4th day of illness and is the first antibody to appear following Dengue infection. IgM antibody gradually increases towards end of first week and it persists for 3 months thereafter. The virus may be at undetectable levels in this period as they tend to be in complex with IgM antibody. Hence NS1 positivity was less during this

period. IgM and IgG combination also showed less positivity (30%) during this period as anti dengue IgG is detectable only at low titer during this period as it appears still later. In a study by **S Datta and Wattal**⁸² IgM detection by MAC ELISA showed 93.6% positivity in early convalescent period.

In more than 10 days duration, IgM & IgG positive with ratio > 1 showed higher positivity (100%) as IgG antibodies would have appeared during this period which increases further in the proceeding weeks and persists lifelong thereafter.

In this study, in secondary dengue, IgM and IgG positive with ratio <1 showed higher positivity (64.4%) than other parameters followed by NS1, IgM and IgG combination (23.7%). As the antibody titres rise extremely rapidly, IgM and IgG are detected more in secondary dengue.

During the acute period of 1-5 days in secondary dengue NS1, IgM and IgG combination showed higher positivity 11(47.8%) than IgM & IgG combination 6 (26.08%), NS1 & IgG 1(4.3%) and IgG alone positive with thrombocytopenia 5(21.7%). The NS1 antigen has reduced sensitivity in secondary dengue because it is sequestered in immune complexes and that target epitopes are not accessible to the plate bound mAb in NS1 ELISA.

High levels of IgG are detectable even in acute phase of secondary dengue due to previous infection and rise dramatically over the proceeding 2 weeks whereas IgM levels tend to be significantly lowered in secondary infection.

In the early convalescent period of 6-10 days in secondary dengue IgM & IgG positive with ratio <1 22 (84.6%) showed higher positivity as IgM to second infection appears during this period. NS1, IgM & IgG showed positivity of 3(11.5%), IgG alone positive with thrombocytopenia 1(3.8%). As IgG is present in both current and past infections, presence of IgG alone does not indicate active infection. Hence it should be combined with platelet count. Only if the platelet count is lowered it indicates current infection. If it is normal, it shows past infection.

In more than 10 days duration of secondary dengue IgM & IgG positive with ratio <1 showed higher positivity (100%) which is due to the presence of IgM & IgG antibodies to the second infection. **Susana vazquez et al**⁸³ 2007 showed that IgM and IgG capture ELISA kits has high sensitivity and specificity and offers good alternative for dengue diagnosis.

ICTs for the detection of NS1, IgM, IgG antibodies have been developed by a number of commercial companies and have found wide applications because of their ease of use and rapidity of results.

The case detection by ICT in this study was 10.2% for NS1, 17.7% for IgM, 15.4% for IgG, whereas in ELISA it was 14.2%, 20.8% and 18.2% respectively. This clearly shows that case detection was more in ELISA. The ICT is found to be less sensitive to ELISA and gives false negative results in some cases. Cross reaction with other flaviviruses gives rise to false positives in some cases. In a study by **RD Kulkarni et al**⁶ 2011 showed NS1 positivity 29%, IgM positivity 50% and IgG positivity 2.8% in ICT.

Masao et al in their study⁸⁴ 2011 in Japan had said that NS1 rapid test is suitable for lab diagnosis due to its reliability and rapidity as it require only 20 minutes for detection. But in view of its low sensitivity it is not suitable for screening purposes.

In a study by **Subhash C Arya**⁸⁵ 2011 the ICT was highly sensitive (88%) and specific (98%).

As diagnostic assays are usually evaluated in terms of Sensitivity and specificity that was calculated by 2x2 cross- tabulation, using a reference

standard (the test most widely used) is compared to determine diagnostic accuracy.

Using ELISA as reference standard, the accuracy indices of the ICT were calculated.

NS1 ICT had a sensitivity of 68%, specificity 99.3%, PPV 94.4%, NPV 85.7%. Using chi-square test p value of <0.001 was obtained which was found to be statistically significant. In a study by **Subhash C Arya et al**⁸⁵ 2011 ICT showed 80-90% detection rate during first 7 days. In a Study by **Vu Ty Han et al**¹⁰, Thailand NS1 LFRT was found to be 100% specific and 72% sensitive in primary than secondary dengue.

In this study the sensitivity and specificity of IgM by ICT was 80.8% & 98.9%, PPV-95.1% & NPV-95.1%. Using chi-square test, p value of <0.05 was obtained for IgM which was found to be statistically significant.

In a study by **M Moorthy et al**¹⁶ 2009 showed that the accuracy indices of ICT for IgM were 81.8%, 75.0%, 61% and 89.6% and concluded that ICT not to be used as stand alone test.

In this study IgG in ICT showed Sensitivity of 81.3%, specificity 98.9%, PPV 94.1% & NPV 96.32% Using chi-square test p value of <0.001 was

obtained which was found to be statistically significant. **Moorthy et al**¹⁶ 2009 study with sensitivity of 87.5%, specificity 66.6%, PPV 72%, NPV 83.9% concluded that ICT with moderate performance could not be used as stand alone test.

WHO's multicentric evaluation of Dengue antibody ICTs had IgM sensitivity from 20% to 100% & specificity from 77% to 98% when compared with reference ELISA.

- Most of the patients were referred from various places and would have received few days treatment before reaching the hospital .The precise day of fever at the time of conducting the test could not be obtained in a large no of cases. In spite of this, NS1 alone was positive in 25% cases in this study.

SUMMARY

- Seropositivity of Dengue in 350 suspected cases during the study period was 30%
- The commonest age group affected by Dengue in this study was 0-10 years in both sexes.
- Dengue was predominant in Males when compared to Females with the ratio of 1.6:1
- Dengue incidence in this study was more during the months of November, December 2011, January, May, June, July of 2012.
- Of all the symptoms, fever was the commonest symptom followed by headache, myalgia, arthralgia, abdominal pain, vomiting, oliguria and rash.
- Melena was the commonest bleeding manifestation followed by bleeding gums, hematemesis and epistaxis in this study.
- Dengue positive cases were classified into Dengue fever (48.5%), Dengue haemorrhagic fever (49.5%) and Dengue shock syndrome (2%) based on WHO guidelines.

- In the haematological parameters, Thrombocytopenia was associated more with Dengue positive cases. Platelet count of 50,000- 1 lakh was commonest range and majority were associated with DHF&DSS.
- WBC count of <4000 was more commonly associated with DHF and DSS
- Elevated Haematocrit level >45 was associated with DHF/DSS.
- Biochemical parameters- AST and ALT were elevated in DF, DHF and DSS.
- Of the USG findings, Gall bladder thickening, pleural effusion and ascites were seen in majority of Dengue haemorrhagic fever and Dengue shock syndrome.
- In this study the incidence of secondary dengue(55.1%) was more than primary dengue(44.9%) with the ratio of 0.8:1
- NS1 positivity was high in primary dengue followed by IgM alone.
- In the acute period of 1-5 days in primary dengue NS1 antigen positivity was found to be higher followed by NS1 & IgM .
- In the early convalescent period (6-10days) of Primary dengue IgM alone had higher positivity followed by IgM & IgG positive with ratio >1.

- In the late convalescent period (10 days) of primary dengue, IgM & IgG positive with ratio >1 alone showed positivity.
- In secondary dengue, IgM & IgG positive with ratio <1 had higher positivity followed by NS1, IgM & IgG combination.
- In the acute period of secondary dengue (1-5 days) NS1, IgM & IgG positive showed higher positivity followed by IgM, IgG positive with ratio <1 , IgG alone positive with thrombocytopenia and lowered NS1 & IgG positivity.
- In the early convalescent period (6-10 days) IgM & IgG positive with ratio <1 showed higher positivity followed by the combination of NS1, IgM, IgG.
- In late convalescent period (>10 days) IgM & IgG positive with ratio <1 alone showed higher sensitivity.
- Case detection rate of ICT was 26.2% and ELISA was 30.5% for 350 cases.
- The percentage of case detection by ICT for all the 3 parameters NS1, IgM and IgG was 10.2%, 17.7% and 15.4% and ELISA showed 14.2%, 20.8% and 18.2%.

- Sensitivity, Specificity, PPV and NPV for NS1 in ICT were 68%, 99.3%, 94.4% and 85.7%.
- Sensitivity ,Specificity ,PPV and NPV of IgM in ICT were 80.8%, 98.9%, 95.1% and 95.1%.
- Sensitivity,Specificity ,PPV and NPV of IgG in ICT were 81.3%, 98.9%, 94.1% and 96.32%.

CONCLUSION

As per the results of this study NS1 ELISA is highly sensitive and proved to be a valuable tool in the diagnosis of dengue particularly in the acute period. NS1 is a reliable marker in primary dengue and when combined with IgM improves the detection rate significantly in a single serum sample in the absence of paired sera.

For diagnosis of Dengue infection irrespective of duration of fever all the three (i.e) NS1, Dengue IgM and IgG by ELISA must be done .This helps to rule out infection and to avoid false negatives. Immuno capture ELISA is highly sensitive in the detection of dengue cases.

Considering the moderate performance of ICT in this study the device cannot be used as a stand alone test and it should always be supplemented by ELISA for the diagnosis of Dengue infection.

Serological differentiation of primary and secondary can be arrived as per the results of the study as shown below

NS1	IgM	IgG	Category
+	-	-	Primary dengue
+	+	-	Primary dengue
-	+	-	Primary dengue
-	+	+	Primary dengue(IgM/IgG ratio>1)
-	-	+	Past infection-(Plateletcount &Total count normal).
-	-	+	Secondary dengue-(Platelet/Total count low)
+	-	+	Secondary dengue
+	+	+	Secondary dengue
-	+	+	Secondary dengue-(IgM/IgG ratio <1)

Significant association of haematological profile and biochemical profile with seropositives in this study insists that these tests should be considered and given importance in the diagnosis and management of dengue.

In conclusion, based upon the higher positivity of the NS1 ELISA both in primary and secondary dengue, this study gains public health importance because it suggests that NS1 antigen should be included along with routinely used antibody testing protocols for early detection and management of cases. ELISA is preferable for diagnostic purposes compared to ICT and the use of

Immunocapture ELISA in the test panel for dengue will reduce the false
Negativity thereby reducing the morbidity and mortality rate.

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APPENDIX - 1

NS1 ELISA

Sample dilution: 75 µl sample diluent + 75 µl of sample (1 in2)

Control dilution: NC&PC diluted in the same ratio.

Calibrator is also diluted in the same ratio as above in triplicate.

Wash buffer (20x): 19 parts of distilled water added to 1 part of wash buffer concentrate. Quantity prepared depending upon the strips used for the test.

IgM ELISA

Serum pre dilution: Suitable test tubes used for dilution of serum, NC, PC, and CO .**Wash buffer dilution:** 19 parts of distilled water is mixed with 1 part of wash buffer concentrate. The required quantity is used and the remaining is discarded

Sample dilution: 5µl Sample diluted with 500µl of sample diluent (1:100)

Control dilution: NC, PC and cutoff calibrator diluted in the same manner as sample.

IgG ELISA

Serum pre dilution:

1. Serum 10 μ l + serum diluent 1000 μ l(1:100 dilution) done using suitable test tubes.
2. Positive control, negative control and calibrators diluted in the same manner

Wash buffer dilution: One Part of buffer concentrate added to 19 parts of distilled water. Total volume prepared as required for the procedure.

APPENDIX-2

Immunochromatography card Test (n=107)

Fever duration	NS1 alone	IgM alone	IgG	NS1+IgM	IgM+IgG	NS1+IgG	NS1+IgM+IgG
1-5 Days(60)	20	4	1	6	11	2	4
6 – 10 Days(36)	0	7	2	1	25	1	0
> 10 Days(11)	0	1	4	0	3	0	0
Total	20	12	7	7	39	3	4

LIST OF TABLES

1.	Seropositivity of Dengue
2.	Age and sex wise distribution of dengue positive cases
3.	Seasonal distribution of dengue positive cases
4.	Clinical presentation of dengue positive cases
5.	Clinical classification based on WHO Guidelines
6.	Platelet count in Dengue positive cases
7.	WBC count of Dengue positive cases
8.	Level of haematocrit in Dengue positive cases
9	Association of platelet, HCT and WBC count with DF,DHF and DSS
10.	ALT and AST levels in Dengue positive cases
11.	USG findings in Dengue positive cases
12.	Serological Categorisation of Dengue positive cases
13.	Primary Dengue based on duration of fever and serological markers.
14.	Secondary dengue based on duration of fever and serological markers
15.	Case detection by ICT and ELISA
15(a)	Case detection by ICT and ELISA for individual parameters
16.	Cross tabulation of ICT and ELISA for NS1
17.	Cross tabulation of ICT and ELISA for IgM
18.	Cross tabulation of ICT and ELISA for IgG

LIST OF CHARTS

1.	Seropositivity of Dengue
2.	Age and sex wise distribution of Dengue positive cases
3.	Seasonal distribution of Dengue positive cases
4.	Clinical presentation of Dengue positive cases
5.	Clinical classification of Dengue positive cases
6.	Association of platelet, HCT and WBC count with DF,DHF and DSS
7.	Serological Categorisation of Dengue positive cases
8.	Primary dengue based on duration of fever and serological markers
9.	Secondary Dengue based on duration of fever and serological markers
10.	Case detection by ICT and ELISA for NS1,IgM ,IgG
11.	Sensitivity ,specificity ,PPV and NPV of ICT for NS1,IgM,IgG

LIST OF COLOUR PLATES

1.	Dengue Duo combo card kit
2.	Dengue NS1 antigen positive
3.	Dengue NS1 antigen with IgM antibody positive
4.	Dengue NS1 antigen with IgM & IgG antibody positive
5.	Dengue NS1 antigen with IgG antibody positive
6.	NS1 antigen negative , IgM & IgG antibody positive
7.	IgM antibody positive
8.	IgG antibody positive
9.	Dengue NS1 ELISA kit
10.	Dengue IgM ELISA kit
11.	Dengue IgG ELISA kit
12.	ELISA reader
13.	Antigen and antibody mixture of IgM & IgG ELISA
14.	Microtitre plate with samples
15.	Microtitre plate with samples after substrate addition
16.	Samples after addition of stop solution

LIST OF ABBREVIATIONS

ADE- Antibody dependent enhancement

ALT- Alanine aminotransferase

AST- Aspartate aminotransferase

Bcell- B lymphocyte

C3a, C5a- complement factors

CFT-Complement fixation test

DEN –Dengue

DENV- Dengue virus

DF- Dengue fever

DHF- Dengue haemorrhagic fever

DSS- Dengue shock syndrome

ELISA- Enzyme linked immunosorbent assay

GB thickening- Gallbladder thickening

HCT- Haematocrit

HAI- Haemagglutination inhibition

HRP-Horse radish peroxidase

IgM- Immunoglobulin M

IgG- Immunoglobulin G

IFN- Interferon

IL- Interleukin

ICT- Immunochromatography

Mab- Monoclonal antibody

NASBA-Nucleic acid sequence based amplification

NT- neutralization

NS1- Nonstructural protein

NPV-Negative predictive value

PPV- Positive predictive value

PD-Primary dengue

RT-PCR- Reverse transcriptase polymerase chain reaction

RANTES- Regulated on activated normal T cell expressed and secreted

SPSS- Statistical package for social sciences

SD-Secondary dengue

T cell- T lymphocyte

TNF- Tumour necrosis factor

TMB- Tetramethylbenzidine

USG- Ultrasonogram

PROFORMA

Date:

Name of the patient:

Age / sex:

Ward:

ID no:

Occupation:

Present address:

Date of admission:

Permanent address:

H/o present illness:

Fever:

- Onset
- Duration
- Type of fever
- Associated with rash/ myalgia/ arthralgia /abdominal pain/vomiting/headache
- Bleeding tendencies

H/O past illness:

- Travel to endemic areas.
- Previous h/o fever with hospitalization
- Living in endemic areas.

Any relevant investigations done outside:

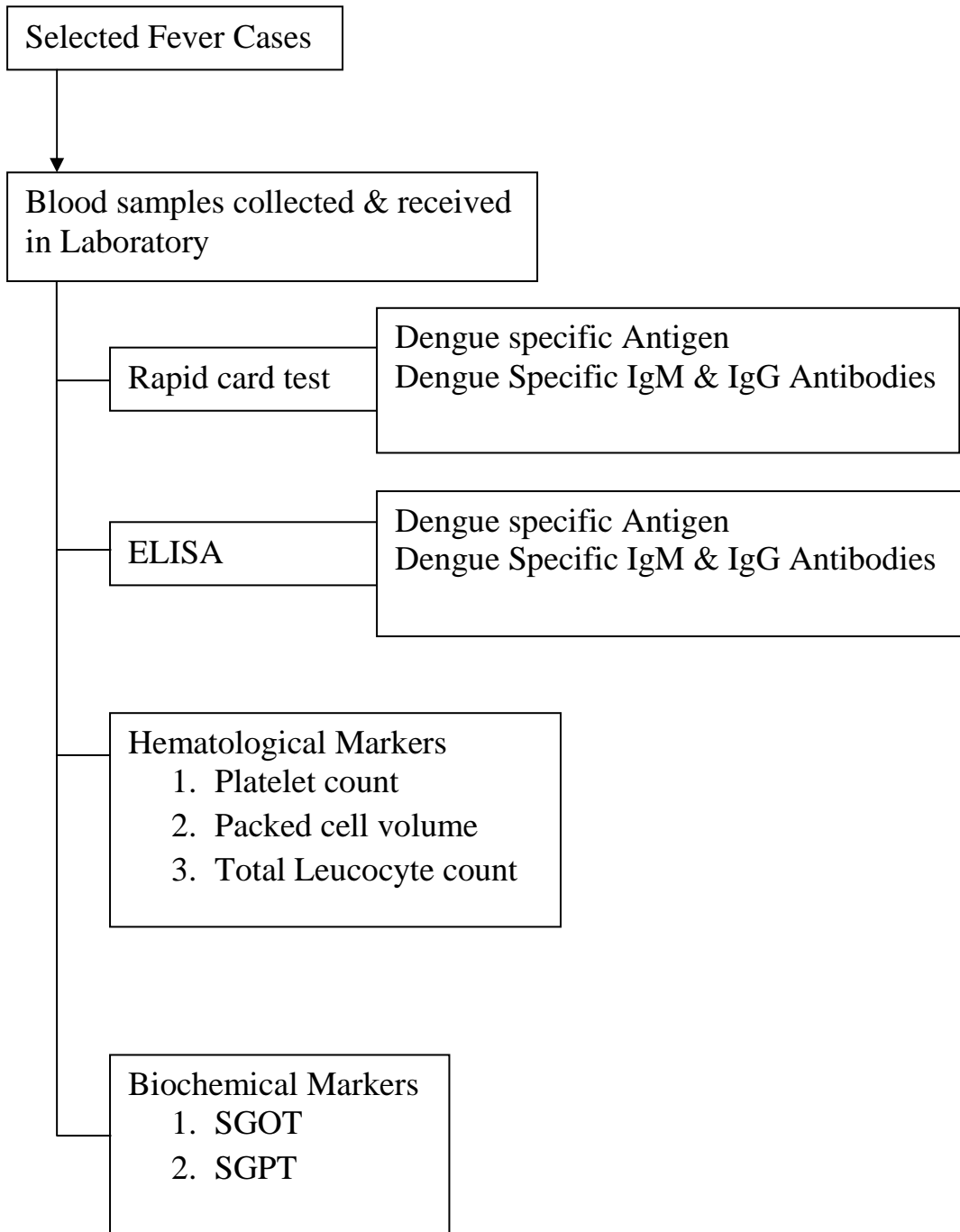
H/O treatment:

Nature of specimen collected:

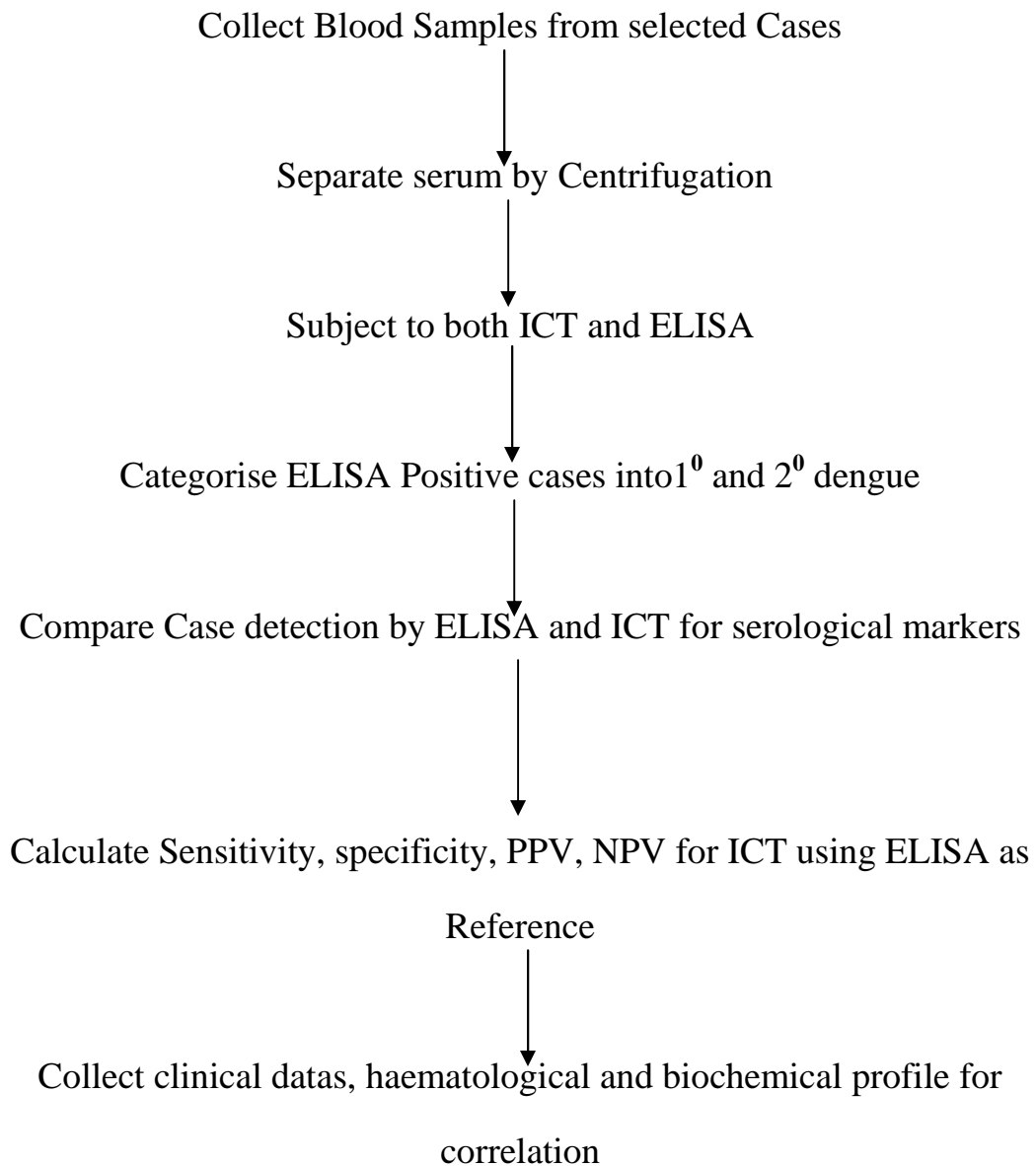
Date of collection:

Investigation done:

PROTOCOL



WORKSHEET



CONSENT FORM

Dr P.Malini, Post Graduate student in the Department of Microbiology, Coimbatore Medical College, Coimbatore, is studying on 'Multivariate Analysis of Dengue in a Tertiary care Hospital' regarding.

I am, _____ of age _____ years, sex - M/F, have been explained about the study. The venepuncture procedure, the risk involved and complications of the procedure were explained to me in my mother tongue. I have also been explained that the data obtained herein may be used for research and publication. I whole heartedly give my consent to participate in the study.

Place:

Date:

Signature

S.No.	IP /OP	Name	Age	Sex	Presenting Complaints	Platelet	WBC	HCT	ALT	AST	USG	ELISA				
												NS1	IgM	IgG		
1	69212	Saraswathi	28	F	Fr - 10 days ,AbdPain 5 days,arthralgia,myalgia	20,000	3000	17.2	28	30	Normal	N	P	P	SEC	DF
2	70201	Karthick	16	M	Fr - 4 days, myalgia,headache	56,000	3000	38.7	27	29	Normal	N	P	P	SEC	DF
3	73576	Rajkumar	33	M	Fr-12 days,Hemetemesis,Haematuria,myalgia,rash	23,000	3200	53.7	52	72	BILPE	N	P	P	SEC	DHF
4	73482	Ganesh	5	mch	Fr - 5days,vomiting,rash	33,000	2000	45.5	25	27	Normal	N	p	P	SEC	DF
5	73486	Sangamithra	4	fch	Fr - 10 days ,Melena 2 days,headache	56000	3500	34.8	21	25	min PE GB thickening	N	p	P	SEC	DHF
6	73561	Elan go	11	mch	Fr - 2 days ,Arthralgia	43000	3000	24	25	27	Normal	P	N	N	PRI	DF
7	73506	Vijaya	60	F	Fr -10days, Altered sensorium,arthralgia,myalgia	89000	16100	32.7	45	89	Grade I MKD	N	p	P	PRI	DF
8	113	Sumaiya	5	fch	Fr - 12DAYS, Melena 3 episodes,rash,epitaxis	43000	3400	25	28	30	GB wall thickning,min.R PE	N	p	P	SEC	DHF
9	351	Divyadharshni	21/2	fch	Fr-7 days,Headache,melena	13000	3600	27.3	97	142	hepatomegaly	N	p	P	SEC	DHF
10		Sanfiya	8	fch	Fr-6 days,Myalgia,vomiting,rash,epitaxis	91000	3000	23.3	31	32	Normal	N	p	P	SEC	DF
11	795	Sanjay	8	mch	Fr-7 days,Abdmn Pain,myalgia	19000	6000	24.7	33	35	GB wall thickening,R PE	N	p	P	SEC	DHF
12	941	Abbas ali	14	M	Fr-1 week,Abdmn Pain 3 days,Vomiting 1 week,Bleeding gums	237000	3100	46	23	27	Mod.ascites	N	p	P	SEC	DHF

13	548	Ajith	10	mch	Fr-6days,Myalgia,bleeding gums	47000	2800	27.6	37	39	Normal	N	p	P	SEC	DF
14	2236	Mohammed Safix	14	M	Fr-3 days,Arthralgia,myalgia	52000	3500	43	41	43	Normal	p	p	P	SEC	DHF
15	2767	Neelakandan	5	mch	Fr-5 days,AbdmnPain 3 days,Oliguria	40000	3000	45.5	43	45	GB thickening	N	p	P	SEC	DHF
16	2512	Gowrav Kumar	11	mch	Fr-6 days,AbdmnPain 2 days,Oliguria 3 days	56000	3600	51.1	47	49	GB thickening	N	p	P	SEC	DF
17	2805	Vanitha	18	F	Fr-5 days, Arthralgia	84000	7000	52	51	53	GB thickening	p	p	P	SEC	DHF
18	2930	Varsha	5	fch	Fr-6 days, Vomiting 3 episode,AbdmnPain,Oliguria	35000	3900	46	55	57	GB thickening,RPE,	N	p	P	SEC	DSS
19	4738	Maran	75	M	AbdmnPain, Fr-2 days,myalgia	136000	2500	46	59	60	Normal	P	p	P	SEC	DF
20	4968	Vijayakumar	20	M	Fr-3 days,myalgia	20000	3100	41.9	49	44	GB wall thickening,R PE	p	N	N	pri	DHF
21	4144	Saraswathy	66	F	Fr-7 days,Arthralgia,myalgia	76000	3600	24	25	23	GB thickening	N	p	P	SEC	DF
22	5765	Jayamani	47	F	Fr-5 days, Melena,puapurahardpala tebleeding	50000	3800	24.6	30	31	Normal	P	N	N	PRI	DHF
23	6163	Samuel	22	M	Fr-2 days,Arthralgia	35000	3950	42.2	27	30	R PE	P	N	N	PRI	DHF
24	61653	Rajendran	27	M	Fr-4 days,Bleeding gums 2 days,myalgia	30000	3800	36.8	27	41	Normal	N	P	P	SEC	DF
25	9143	Haridharshini	4	fch	Fr,Vomiting 6 days,oliguria	83000	2000	38.2	28	25	0	N	p	P	SEC	DF
26	18828	Ansiya	8	fch	Fr-11 days,Vomiting 1 day	53000	3100	38.8	29	22	GB thickening	N	P	P	SEC	DHF

27	11348	Nuthun	26	M	Fr-5 days,Vomiting,Arthralgia, Haematuria,Epitaxis	36000	2600	48.6	45	50	R minimal PE	P	N	N	PRI	DHF
28	11943	Velmurugan	32	M	Fr-4 days,arthralgia,myalgia,b leeding gums	39000	2000	45.7	22	24	Normal	P	N	N	PRI	DF
29	68327	Irfanahmed	6	mch	Fr-6 days,Myalgia	33000	3910	36	25	26	Thickened GB,,Bil.PE	N	P	P	SEC	DHF
30	72407	Priyanka	8	fch	Fr-5 days,arthralgia	31000	3700	38.5	41	43	-	p	N	N	PRI	DF
31		Anandkumar	14	M	Fr-5days,Hemetemesis,myalgia	55000	3000	40	42	40	Normal	P	N	N	PRI	DF
32	1E+05	Mansurali	24	M	Fr-7 days,Haematuria	66000	3000	45.5	35	30	GB wall thickening	p	p	P	SEC	DHF
33	18433	Sagana	5	fch	Fr-6 days,myalgia	82000	6000	46	25	27	minimal R pleural effusion	p	p	P	SEC	DHF
34	19258	Bathri	3	fch	Fr-11days, Edima 2 days,hemetemesis,rash	48000	3500	46.4	20	25	GB wall thickening	N	p	P	SEC	DHF
35	9676	Karthick	3	fch	Fr-4 days,abd.pain	74000	8000	48	58	55	diffuse GB thickening	p	N	P	SEC	DHF
36	20502	Vignesh	6	mch	Fr-4 daysvomiting,maylgia	28000	8200	45.8	26	21	minimal R PE,GB wall thickening	p	P	N	PRI	DHF
37	20432	Rahul	3	mch	Fr-6days,vomiting	27000	4900	37.5	20	25	minimal R PE,GB wall thickening	N	p	P	SEC	DHF
38	24426	Vinithkumar	3	mch	Fr-7 days, Abdmn Pain,Nausea Vomiting	17000	3100	36	26	28	GB wall thickening	N	p	P	SEC	DHF
39	24481	Prahalad	30	M	Fr-6 days,myalgia	49000	4000	48	43	47	Normal	N	p	P	SEC	DHF

40	26097	Harichander	2	mch	Fr-8 days, Abdmn Pain,	60000	5000	33	35	30	-	N	p	P	SEC	DF
41	2E+05	Ashok kumar	17	M	Fr-8 days,arthralgia,myalgia	37000	3500	42	37	30	GB thickening	N	p	P	SEC	DHF
42	35207	Balu	36	M	Fr-4 days, Arthralgia	45000	3800	33.1	43	45	Hepatomegaly	P	p	P	SEC	DF
43	35906	Ramesh	38	M	Fr-7 days, Vomiting 1 episode,Myalgia,bleeding gums	20000	10600	48.5	28	29	GB wall thickening	P	p	P	SEC	DHF
44	32911	Manoba	9	mch	Fr-5 day Abdmn Pain, Vomiting,headache,mele na	37000	8200	41	25	27	GB thickening	N	p	P	SEC	DHF
45	32910	Jeevasri	10	fch	Fr with Chills,Abdmn Pain 4days,myalgia	31000	2800	33.7	29	25	Bil.PE,min.FF abdomen	P	N	N	PRI	DHF
46	32075	Meenambigai	6	fch	Fr-4 days,vomiting,myalgia	26000	3200	37.2	28	21	GB wall thcickened	P	p	P	SEC	DHF
47	7815	Kumaravel	7	mch	Fr-7 days,vomiting,myalgia	49000	4500	32	27	28	Normal	N	P	P	SEC	DHF
48	7816	Vetrivel	4	mch	Fr-7 days,Abdmn pain,Vomiting	55000	3200	40	23	25	normal	N	P	P	SEC	DF
49	33544	Arunvijay	15	M	Fr-11days, abd.pain,myalgia	17000	3100	42	36	32	min.RPE,Asscites	N	P	P	SEC	DHF
50	35493	Damodaran	12	M	Fr-12 days,Myalgia,Arthralgia	129000	2200	33.6	27	24	Gb wall edematous	N	P	P	SEC	DF
51	35095	Saranya	16	F	Fr-4 days,artralgia	17000	5700	32.2	45	48	GB edematous,Bil.PEFF abdomen &pelvis	P	N	N	PRI	DHF
52	35184	sathish	24	M	Fr-4 days,Myalgia,Arthralgia,h eadache,vomiting	46000	5000	52.5	30	32	GB wall edematous	p	p	N	PRI	DF

53	35240	Ashok kumar	8	M	Fr-6days,Vomiting 5 days,Melena,Abdmn Pain	36000	3200	33.7	35	30	GB wall edematous ,Bil.PE,ascites	N	p	P	SEC	DHF
54	35375	Valliyammal	60	F	Fr-5 days, Arthralgia	35000	7000	35	32	30	GB contracted,	P	N	N	PRI	DF
55	35376	Mohammedhussain	15	M	Fr-11days,myalgia	17000	3800	50.8	45	48	GB thickening,	N	p	P	SEC	DHF
56	31346	Murugan	32	M	Fr-4 days,dysuria,headache,arthralgia	61000	2600	42.5	29	30	-	P	p	N	PRI	DF
57	35333	Navanethan	4	mch	Fr-11 days,vomiting,myalgia	45000	3500	40.2	28	30	GB thickened	N	P	P	SEC	DF
58	35236	Selvakrishnan	1	mch	Fr-4 days,headache	56000	14000	39.5	32	80	NA	p	P	P	SEC	DF
59	33510	Logeswaran	19	M	Fr-7 days, Myalgia,arthralgia	24000	5100	19	25	22	Normal	N	p	P	SEC	DF
60	32336	Manikandan	32	M	Fr-1 weeks,Arthralgia 10 days	87000	3000	36	42	45	NA	N	p	P	SEC	DF
61	34418	Shankar	26	M	Fr-2 days,Myalgia,arthralgia	7000	3000	49.4	24	26	Hepatomegaly	p	P	P	SEC	DF
62	34579	Sivakumar	20	M	Fr-12 days, Vomiting,Melena 3 days	14000	2900	45.6	28	30	minimal R PE ,Ascites	N	P	P	SEC	DHF
63	36503	Dharshini	7	fch	Fr-5 days,Refusal of feeds 3 days,melena 1 day	55000	13400	27.6	32	40	Hepatomegaly	N	N	P	SEC	DF
64	36331	Jithin Samuel	10	mch	Fr-4 days,arthralgia	87000	330000%	33	37	35	hepatomegaly, GB contracted	N	N	P	SEC	DF
65	36450	Divya	21	F	Fr-5days, rash, Abdmn pain	88000	3600	45.9	35	32	GB thickened	P	N	N	PRI	DHF
66	36394	Vijayaraghavan	1.5	mch	Fr-5 days,Abdmn Pain 4 days	360000	3300	48.8	42	43	GB contracted,	P	N	N	PRI	DHF

67	36436	Bhagyalakshmi	18	F	Fr-5 days, Vomiting 3 days, Abdmn pain 4 days	68000	3600	46.9	30	42	GB wall thickening	P	N	N	PRI	DHF
68	32482	Amirtha	17	F	Fr-10 days, arthralgia	200000	6500	31	25	27	normal	N	P	N	PRI	DF
69	34794	Amirtham	40	F	Fr-5 days, arthralgia, myalgia	100000	4000	42	43	45	NA	P	N	N	PRI	DF
70	35040	Priyadharshini	12	fch	Fr-5 days, arthralgia	111000	4800	12.7	25	27	hepatomegaly	P	N	N	PRI	DF
71	37045	Deepika	17	F	Fr-3 days, arthralgia	68000	6200	22.5	26	25	normal	P	N	N	PRI	DF
72	33204	Kelvin	46	M	Fr, Abdmn pain 3 days, myalgia	70000	2500	32	23	25	-	p	N	N	PRI	DF
73	35331	AyishaRithiga	23	F	Fr-3 days, Arthralgia	100000	1500	12.7	25	25	Normal	P	N	N	PRI	DF
74	34531	Munna	23	M	Fr-6 days, vomiting, abdmn pain, arthralgia	16000	6300	33.7	29	27	Normal	N	N	P	SEC	DF
75	2E+05	Dharshan	1	mch	Fr-5 days, Abdmn pain, hemetemesis	65000	2500	47	50	53	GB thickening, pleural effusion, ascites.	p	N	p	SEC	DSS
76	78957	Kamala	22	F	Fr-10 days, Myalgia	27000	3400	32.8	26	21	Normal	N	P	N	PRI	DF
77	73621	Amudhavalli	22	F	Fr-3 days, Myalgia, Arthralgia	13200	4800	36.9	25	28	NA	P	p	P	SEC	DF
78	72846	Yamuna	6	fch	Fr-5 days, vomiting	113000	5600	28	24	26	,R PE	p	P	N	PRI	DHF
79	7973	Aravivel	12	mch	Fr-5 days, Myalgia, Abdmn pain, Melena	45000	6000	34	42	43	GBthickening	N	p	P	SEC	DHF
80	7862	Kalingaraj	13	M	Fr-7 days, vomiting	39000	2400	46.6	35	37	GB thickening	N	p	P	PRI	DHF

81	70488	Jaya	21	F	Fr-3 days,	63000	1700	38.5	32	39	Normal	N	p	N	PRI	DF
82	7389	Mani	34	M	Fr-6 days,Myalgia,Nausea,Vo miting	14000	3000	35	29	27	-	N	p	P	PRI	DF
83	23275	Nitesesh	6	mch	Fr-10 days	94000	6000	36	28	29	-	N	p	N	PRI	DF
84	71821	Dinakaran	8	mch	Fr-15 days,Abdmn Pain 3 days	190,000	9800	30	25	27	Normal	N	p	P	PRI	DF
85	62920	Poongodi	7	fch	Fr-5 days,Abdmn pain,	82000	2300	38.6	27	29	Normal	P	N	N	PRI	DF
86	7288	Maheswari	10	fch	Fr-3 days,myalgia	70000	3000	49.7	28	30	hepatomegaly, PE	P	p	N	PRI	DHF
87	32266	Lakshmanan Babu	8	mch	Fr-5 days	52000	2000	39	29	30	Normal	P	N	N	PRI	DF
88	7173	Umadevi	9	fch	Fr-3 days,Melena 1 day, Oliguria 2 days	34000	1700	48	25	27	FF abdomen	P	p	P	SEC	DHF
89	31102	Priya	23	F	Fr-3 days,	36000	3000	35	28	30	-	P	N	N	PRI	DF
90	28551	lalitha	15	F	Fr- 5 days,	72,000	2000	38	25	27	Normal	P	N	N	PRI	DF
91	32394	Sathish Kumar	38	M	Fr-5 days,Vomiting	60000	5200	46	30	42	minimal R PE	P	N	N	PRI	DHF
92	67696	Sikinder	35	M	Fr-5 days,Abdmn Pain 2 days,arthralgia	37000	3000	29.4	25	27	Normal	P	p	N	PRI	DF
93	68299	Indurekha	13	F	Fr - 3 days,arthralgia	57,000	7000	30	33	35	-	P	p	N	PRI	DF
94	71223	Venkatprabu	8.12	mch	Fr-7 days,Melena 2 days,abdominal pain	29000	3000	45.3	25	29	bil.PE	N	p	N	PRI	DHF

95	3320	Gopalakrishnan	10	mch	Fr-5 days,Vomiting 3 days, Melena 3 days	73000	3000	35	27	29	ascites	P	N	N	PRI	DHF
96	31647	Venkatachalam	30	M	Fr-4 days, Abdmn Pain, Giddiness,arthralgia	15000	3000	40	29	30	-	P	N	N	PRI	DF
97	30889	Bharath	10	mch	Fr-5 days, Abdmn Pain,Melena	80000	3800	46	27	29	pleural effusion	P	p	P	SEC	DHF
98	3E+05	Karthick	11	M	Fever 5 days, Headache 5 day,Melena 4 days, Abdmn Pain	32000	2600	37.5	30	35	bil.PE	P	p	P	SEC	DHF
99	31346	Murugesan	32	M	Fr-6 days, Abdmn Pain,Dysuria 4 days	61000	2600	45.5	31	35	pleural effusion	N	p	N	PRI	DHF
100	31344	Ashok	10	mch	Fr-4 days, Vomiting,Poor appetite,Myalgia, Abdmn pain	35000	3100	57.3	32	30	ascites	P	N	N	PRI	DHF
101	33233	Kaviraj	16	M	Fr-7 days Abdmn pain,arthralgia	73000	2700	42	20	25	pleural effusion	N	p	P	PRI	DHF
102	34282	Dilfar	5	mch	Fr-7 days,Vomiting 2 days	22000	3500	46.5	25	27	minimal R PE	N	p	N	PRI	DHF
103	28819	sakthivel	27	M	Fr-6 days,Myalgia	48000	2900	39	23	25	-	N	p	N	PRI	DF
104	33367	Amritha Iniya	7	fch	Fr-3 days, Vomiting, Abdmn pain	21000	5600	39.8	43	47	ascites	N	N	P	SEC	DHF
105	33739	Thangomani	40	F	Fr-2 days,Burning Micturition,Myalgia	31000	2400	36	25	24	normal	p	p	N	PRI	DF
106	33755	Subramani	62	M	Fr-12days, Myalgia	57000	3000	33	25	27	normal	N	P	P	SEC	DF
107	33855	NavaneethaKrishnan	12	mch	Fr-5 days,Myalgia,Abdmn pain,Melena	28000	3500	48	29	30	ascites	N	N	P	SEC	DHF

Note: P-Positive, N - Negative, M-Male,F-Female,mch-MaleChild,fch-FemaleChild

Multivariate Analysis of Dengue in a Tertiary Care Hospital.

ABSTRACT

Background: Dengue is a viral infection with potential fatal complications. It is considered as one of the world's major emerging tropical diseases. More than 2.5 billion people are at risk of infection and more than 100 countries have endemic transmission. The emergence and reemergence of dengue is attributed to demographic and societal changes with increased movement of people from endemic areas and inadequate mosquito control measures. It is an emerging disease in Tamilnadu. Dengue virus is an enveloped ssRNA virus of 45-50 nm belonging to Genus flavivirus and Family flaviviridae. Dengue is transmitted by *Aedes aegypti* mosquitoes. It causes wide spectrum of illness ranging from undifferentiated illness to Dengue haemorrhagic fever and Dengue shock syndrome. Specific IgM and IgG detection has been the mainstay of diagnosis for a long time. Dengue specific antigen is found to be highly specific and reliable marker from the first day of diagnosis. **Aim and objectives:** To study the multivariate analysis of Dengue with serological differentiation of primary (PD) and secondary dengue (SD), comparative evaluation of Immunochromatography (ICT) with Immunocapture Enzyme Linked Immuno Sorbent Assay (ELISA) and special emphasis on Nonstructural protein (NS1) antigen as an early diagnostic marker. To correlate Dengue clinically with haematological and biochemical profile. **Materials and methods:** After taking informed consent, blood was drawn from three hundred and fifty suspected cases and were subjected to both ICT and ELISA. ELISA positive cases were analysed further. Accuracy indices of ICT were calculated using ELISA as reference assay. **Results:** Out of 350 samples tested, 107 were positive by ELISA (30.5%) for one or more serological markers. The common age group affected were 0-10 years in both sexes with male: female ratio of 1.6:1. Incidence was high during November to December 2011 & January, May

,june & July 2012. Clinically seropositives were classified as Dengue fever, Dengue haemorrhagic fever and Dengue shock syndrome. There was significant association of thrombocytopenia, HCT and WBC count with Dengue fever, DHF and DSS. The 107 seropositives by ELISA were categorized as primary and secondary dengue based on duration of fever and serological markers. Out of 107, 27 were positive for NS1 only, 9 were positive for IgM alone, only 6 showed IgG alone. More than one marker was detected in the remaining 65 samples. In 48 primary dengue cases NS1 had higher positivity and in secondary dengue IgM&IgG ratio >1 had higher positivity. Sensitivity, specificity, PPV and NPV of the ICT were calculated using ELISA as reference for individual markers which showed moderate performance. Haematological and biochemical profile also showed statistical significance in association with Dengue fever, DHF and DSS. **Conclusion:** Inclusion of NSI in the routine diagnosis of dengue increases the detection rate of cases significantly in the early period. Irrespective of fever duration, all the three ELISA must be done to rule out Dengue infection like NS1, IgM, and IgG. Immunocapture ELISA is reliable and sensitive than ICT even though it is highly specific. Haematological and biochemical tests should also be given importance along with serological markers for dengue diagnosis and management.

Keywords: *Dengue, NS1, PD, SD, ICT, Immunocapture ELISA.*