COMPARISON OF RAPID IMMUNOCHROMATOGRAPHIC METHOD AND ELISA FOR THE DETECTION OF NS1, IgM, IgG FOR THE DIAGNOSIS OF DENGUE VIRUS INFECTION

By

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In

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Under the guidance of

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Date: **Dr. RONNI MOL P**

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ABSTRACT

Introduction :

Dengue is one of the rapidly emerging global threats. Many outbreaks are being noticed nowadays all around the world. In situations of epidemics and routine cases, early diagnosis is the key to successful management of dengue cases. Many diagnostic kits are available commercially for the same purpose. But their validity is unknown. The standard is ELISA, though it is time consuming. In the present study, the test results of commercially available rapid immunochromatographic card test (Dengue Day 1 Kit, J.Mitra) is compared with ELISA(J. Mitra) as the standard.

Materials & methods:

The study was conducted from December 2012 to August 2014 at Shri B M Patil Medical College & Research centre, Bijapur. Probable dengue cases were diagnosed as per the WHO criteria and rapid immunochromatographic card test and ELISA were conducted on the same serum sample for the detection of NS1 antigen, IgM & IgG antibodies. Results were analyzed.

Results:

A total of 90 probable dengue cases were selected. 36 (40%)cases were found to be positive for dengue rapid immunochromatographic test, whereas 39(43.3%) cases were found to be dengue positive by ELISA. The sensitivity of rapid test was 92.31% & specificity was 100% along with positive predictive value of 100% and negative predictive value of 94.4% and was compared with other studies.

Conclusion:

The study shows that the sensitivity(92.31%) of rapid card test is less but has a good specificity(100%). Thus the card test can be used for screening but with the support of ELISA. Highly suspicious cases should be subjected to investigations with higher sensitivity & specificity, though the results take more time.

MeSH Keywords:

Dengue, Immunochromatography, antibody, NS1 protein, dengue virus.

LIST OF ABBREVIATIONS

ADE	Adverse Drug Events
Abs	Antibodies
DEN1	Dengue virus type 1
DEN2	Dengue virus type 2
DEN3	Dengue virus type 3
DEN4	Dengue virus type 4
DF	Dengue Fever
DHF	Dengue Haemorrhagic Fever
DIC	Disseminated Intravascular Coagulation
DSS	Dengue Shock Syndrome
ELISA	Enzyme Linked Immunosorbent Assay
MAC-ELISA	IgM Antibody Capture ELISA
NS1	Non structural antigen 1
NVBDCP	National Vector Borne Disease Control Programme
RTPCR	Reverse Transcriptase – Polymerase Chain Reaction
WBC	White Blood Cells
WHO	World Health Organization
w.r.t	with respect to

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INTRODUCTION

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

-Benjamin Rush (1745-1813)

Dengue fever is an important arthropod borne viral disease of public health significance. In recent decades the global prevalence has grown dramatically with estimated 2.5 billion people at risk of acquiring dengue viral infection and more than 50 million new infections projected annually.[1]

The Asia Pacific Dengue Strategic Plan for both regions (2008--2015) has been prepared in consultation with member countries and development partners in response to the increasing threat from dengue, which is spreading to new geographical areas and causing high mortality during the early phases of outbreaks. The strategic plan aims to aid countries to reverse the rising trend of dengue by enhancing their preparedness to detect, characterize and contain outbreaks rapidly and to stop the spread to new areas.[2]

In 2013, dengue ranks as the most important mosquito-borne viral disease in the world. The emergence and spread of all four dengue viruses ("serotypes") from Asia to the Americas, Africa and the Eastern Mediterranean regions represent a global pandemic threat. Although the full global burden of the disease is still uncertain, the patterns are alarming for both human health and the economy.[3]

According to World Health Organisation estimates, the incidence of dengue disease has increased by a factor of 30 over the past 50years.[4]

From being a sporadic illness, epidemics of dengue have become a common occurrence worldwide. Dengue fever and dengue hemorrhagic fever is endemic in areas of South East Asia i.e. Bangladesh, India, Indonesia, Maldives, Myanmar, Srilanka and Thailand. Dengue is a major cause of hospitalization and death, especially among children in these regions. India is endemic for DF and DHF. All the four serotypes are found in the country. Case fatality rates in endemic countries are 2.5%.[5] During epidemics of dengue, attack rates among susceptibles are 40-90%.[6]

During the past five decades, the incidence of dengue has increased 30-fold. Some 50–100 million new infections are estimated to occur annually in more than 100 endemic countries, with a documented further spread to previously unaffected areas; every year hundreds of thousands of severe cases arise, including 20,000 deaths; 264 disability-adjusted life years per million population per year are lost,[7] often affecting very poor populations. The true numbers are probably far worse, since severe underreporting and misclassification of dengue cases have been documented.[8]

An increased disease burden has been linked to the resurgence of mosquito vector Aedes aegypti, overcrowding, urbanization and increasing travel. Despite its

significant health and economic impacts, as of yet there is no specific treatment or therapy for dengue infection and the outcome depends on medical care provided by the doctor to the patient.[9]

Dengue virus is a flavivirus which is enveloped, single stranded positive sense RNA virus. The genomic RNA is 11 kb long and contains 10 genes encoding 3 structural proteins capsid(C), membrane(M), an envelope(E) and seven non structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5).

Dengue symptoms range from mild fever, the most common form, to potentially fatal dengue haemorrhagic syndrome(DHF) and dengue shock syndrome(DSS) or encephalitis and hepatitis.[4]

In view of high mortality rate and to reduce the disease burden, it is imperative to have a rapid and sensitive laboratory assay for early diagnosis of dengue fever.

At present, laboratory confirmation of dengue infection is done by following methods,

1. Dengue virus isolation.
2. Viral RNA detection by reverse transcription polymerase chain reaction.
3. Detection of dengue NS1 antigen.
4. Detection of dengue specific antibodies IgM and IgG by enzyme linked immunosorbent assay (ELISA).

Virus isolation by cell culture and subsequent detection by immunofluorescence, though the gold standard,[10,11] cannot be used as routine diagnostic procedure due to its low sensitivity, laborious procedure and time consumption**.** On the other hand, RT-PCR and MAC-ELISA are expensive, and

usually take at least half a day for running the assay. Moreover, these facilities are not widely available in the clinics and hospital settings.[12]

Also the MAC ELISA, which is commonly used assay, has a low sensitivity in four days of illness. However, it has the advantage of frequently requiring only a single, properly timed blood sample.[13]

The detection of secreted NS1 protein holds promise in early diagnosis of dengue infection especially within first four days of illness. When used in combination with MAC ELISA on a single sample it significantly improves the diagnostic algorithm without the requirement of paired sera.[10]

A rapid test kit would be useful to provide early diagnosis of acute dengue infection. The advantage of this kit is that it is designed to detect both dengue virus NS1 antigen and differential IgM/IgG antibodies to dengue virus in human blood.[12]

The rapid test does not involve any specific laboratory equipments except micro centrifuge for serum separation. So the rapid test may prove to be useful aids in screening, in clinical diagnosis of dengue infection, more so in the resource poor peripheral health setting. It can prove to be a useful tool to hasten the initiation of the first line of the management and thereby can be great help to the health care providers in the rural area.[14]

Hence this study was carried out to evaluate the performance of a rapid immunochromatographic test device for the detection of NS1, IgM, and IgG response in comparison with enzyme linked immunosorbent assay (ELISA) for NS1, IgM and IgG detection for the diagnosis of dengue infection.

An accurate clinical assessment of all included cases was also performed during the study, since it was necessary for the proposed case definition of suspected dengue patients.

AIMS &OBJECTIVES OF THE STUDY

1. Screening the blood samples for dengue virus infection in suspected patients.

2. To compare rapid immunochromatographic test assay with ELISA for detection of NS1 antigen, IgM & IgG antibodies for diagnosis of dengue infection.

REVIEW OF LITERATURE

HISTORY

The word "dengue" is derived from the Swahili phrase Ka-dingapepo, meaning "cramp-like seizure".[15] Dengue fever was first referred as "water poison" associated with flying insects in a Chinese medical encyclopedia in 992 from the Chin Dynasty (265-420 AD).

The first clinically recognized dengue epidemics occurred almost simultaneously in Asia, Africa, and North America in the 1780s.

The first clinical case report dates from 1789 of 1780 epidemic in Philadelphia is by Benjamin Rush, who coined the term "break bone fever" because of the symptoms of myalgia and arthralgia. The term dengue fever came into general use only after 1828.[13]

It is speculated that DENV was the etiological agent during disease outbreaks in the French West Indies in 1635, in Panama in 1699, and Philadelphia epidemic of 1780.

Thus, dengue or a very similar illness had a wide geographic distribution before the 18th century, when the first known pandemic of dengue-like illness began.[13]

In 1823 the term dinga (dyenga) was used for the first time to designate a disease in Zanzibar.[17]

In 1906 Thomas Lane Bancroft suggested that Aedes aegypti mosquito was involved in the transmission of Dengue.[18]

In 1907 Percy Ashburn and Charles Franklin Craig were the first to prove that the etiological agent of Dengue was a filterable virus, the 2nd human viral disease after yellowfever to be identified.[17]

In 1939-1945 during world war II the ecologic disruption facilitated the increased transmission of mosquito borne diseases and multiple dengue virus strains became endemic (hyperendemic) in South East Asia and pacific, also an increased incidence of complicated dengue emerged.[19]

1944-45 Albert B Sabin and co-workers isolated a number of dengue virus strains by inoculating infectious human sera into human volunteers.[20]

The first major epidemic of DHF occurred in 1953-54 in Phillipines followed by a quick global spread of epidemics of DF/DHF.

2000 – from past 50years dengue incidence has increased dramatically and at present it is endemic in more than 100 countries.[21]

In 2011 – outbreak was seen in Brazil, Pakistan, Paraguay and Thailand. Record dengue outbreaks was reported in 2013 in many countries: Brazil (double deaths over 2012), Singapore, and Thailand (worst in 20 years), among others. San Pedro Sula, Honduras declared a dengue 'state of emergency', while Central America had its worst outbreaks since 2008. Dengue deaths have tripled in Malaysia until Feb 2014 over 2013, after tripling 2013 over 2012.[22]

In 2014, trends indicate increases in the number of cases in the Cook Islands, Malaysia, Fiji and Vanuatu, with Dengue Type 3 (DEN 3) affecting the Pacific Island countries after a lapse of over 10 years.[23]

PLACE	YEAR	DENGUE SEROTYPES
INDONESIA	1983	1,2,3,4
THAILAND	2000	1,2,3,4
PERU	2002	1,2,3
NICARAGUA	2006	1,2
HONG KONG	2008	1,2,3,4
MARTINIQUE	2008	2,4
TAIWAN	2009	2,3
THAILAND	2010	1,2,3,4
INDIA	2010	1,2,3,4
VIETNAM	2010	1,2,3,4

Epidemiology of dengue in India

The first epidemic of clinical dengue-like illness was recorded in Madras (now Chennai) in 1780 and the first virologically proved epidemic of DF in India occurred in Calcutta and Eastern Coast of India in 1963-1964.[24]

The very first report of existence of dengue fevers in India was way back in 1946.[25] In 1963-64 an initial epidemic of DF was reported on the eastern coast of India, it spread northwards and reached Delhi in 1967 and Kanpur in 1968.[26] Simultaneously it involved the southern part of India and gradually whole country with wide spread epidemics followed by endemic/ hyperendemic prevalence of all the four serotypes of DV.

The epidemic at Kanpur during 1968 was due to DV-4 and during 1969 epidemic both DV 2 and DV4 were isolated. It was completely replaced by DV2 during epidemic in the adjoining city of Hardoi.[27]

DV 2 was isolated during the epidemics of Dengue in urban and rural areas of Gujarat state during 1988-89. Outbreaks of DF occurred in Rajasthan by DV1 and DV3, Madhya Pradesh by DV3, Gujarat by DV2 and in Haryana by DV2.[28]

DV 2 was predominant serotype circulating in Northen India including Delhi, Lucknow and Gwalior[29] while DV1 was isolated during 1997 epidemic at Delhi.[30]

The phylogenetic analysis by the Molecular Evolutionary Genetics Analysis programme suggests that the 1996 Delhi isolates of DV-2 were genotype IV. The 1967 isolate was similar to a 1957 isolate of DV-2, from India, and was classified as genotype V. This study indicates that earlier DV-2 strains of genotype V have been replaced by genotype IV.[31]

The Gwalior DV-2 viruses were classified into genotype-IV, and were most closely related to Delhi 1996 DV-2 viruses and FJ 10/11 strains prevalent in the Fujian State of China. Two earlier Indian isolates of DV-2 were classified into genotype-V. Genotype V of DV-2 has been replaced by genotype IV during the past decade, which continues to circulate silently in north India, and has the potential to re-emerge and cause major epidemics of DF and DHF. DV-2 has also been reported from southern India - in Kerala alongwith DV-3.[32]

DV-3 has been isolated during the epidemics at Vellore in 1966 at Calcutta in 1983and in 1990, at Jalore city, Rajasthan in 1985 at Gwalior in 2003 and 2004and at Tirupur, Tamil Nadu in 2010.[33] Phylogenetic analysis showed that the Madurai isolates were closely related to Gwalior and Delhi isolates. The emergence of DV-4

has been reported in Andhra Pradesh[45]and Pune, Maharashtra[34], which was also implicated in increased severity of disease.

At Delhi, till 2003, the predominant serotype was DV-2 (genotype IV) but in 2003 for the first time all four dengue virus subtypes were found to co-circulate in Delhi thus changing it to a hyperendemic state followed by complete predominance of DV serotype 3 in 2005. [35]During the 2004 epidemic of DHF/DSS in northern India a sudden shift and dominance of the DV serotype-3 (subtype III) occurred replacing the earlier circulating serotype-2 (subtype IV).[36]

Co-circulation of DV serotypes in Delhi in 2003-2004 has also been reported, which may have implications for increased DHF/DSS. Emergence of a distinct lineage of DV-1, having similarity with the Comoros/Singapore 1993 and Delhi 1982 strains, but quite different from the Delhi 2005 lineage and microevolution of the pre-circulating DV-3 has been reported.[37]

Co-circulation of several serotypes of dengue viruses has resulted in concurrent infection in some patients with multiple serotypes of DV. Further, replacement of DV-2 and 3 with DV-1 as the predominant serotype in Delhi over a period of three years (2007-2009) has been reported. Concurrent infection by Chikungunya and DV-2 was reported from Velloreand Delhi.[38]

DV-1 was isolated in 1956 at Vellore. All the Indian DV-1 isolates belong to the American African (AMAF) genotype. The Indian DV-1 isolates are distributed into four lineages, India I, II, III and the Africa lineage. Of these, India III is the oldest and extinct lineage; the Afro-India is a transient lineage while India I is imported from Singapore and India II, evolving in situ, are the circulating lineages.[39]

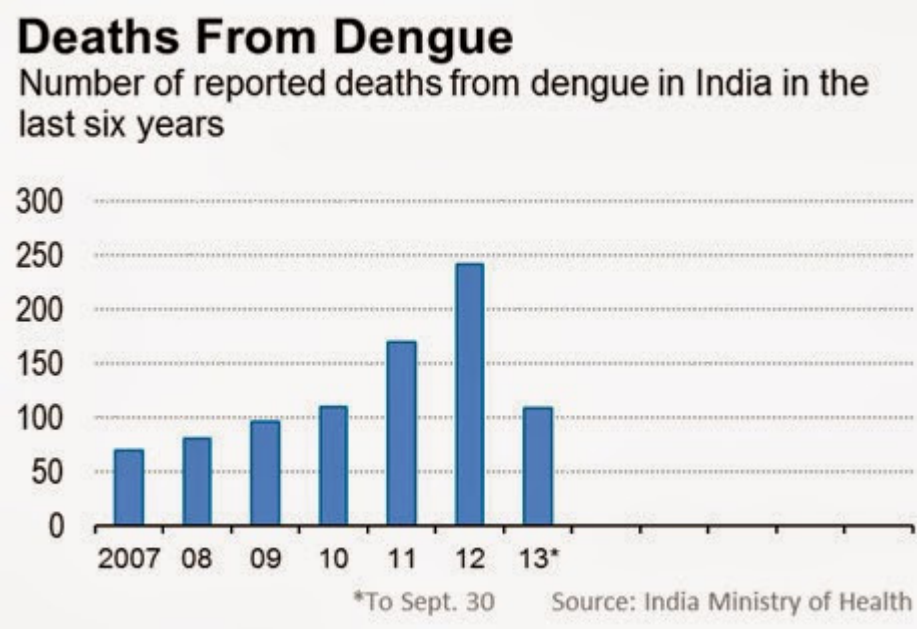


Fig 1: Deaths from dengue

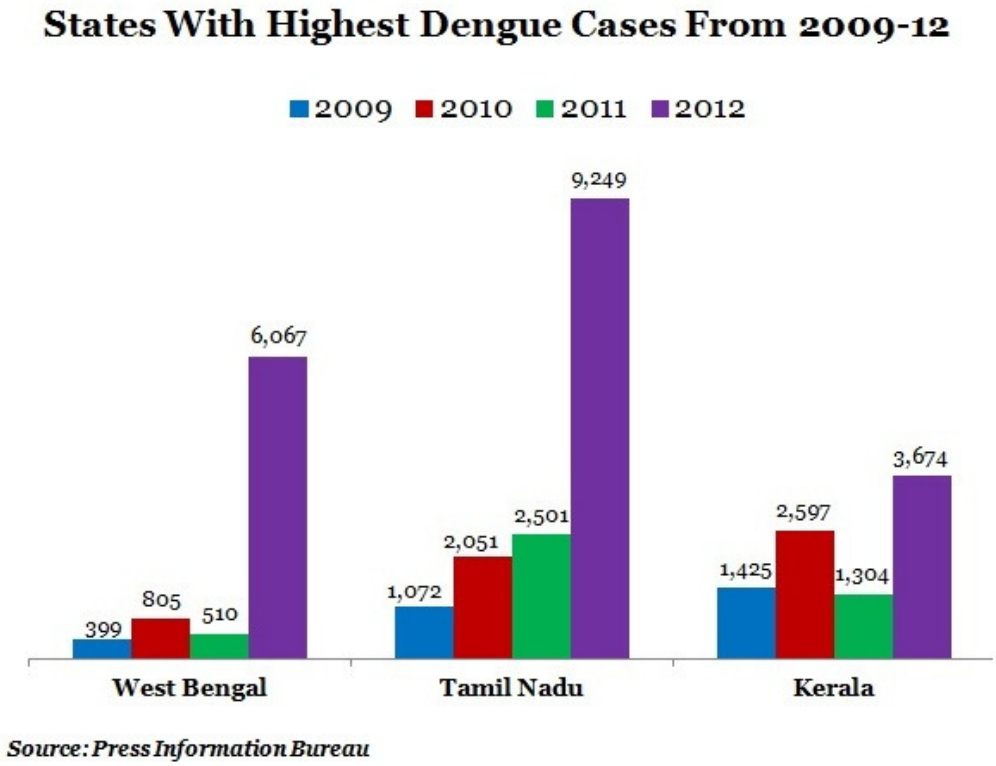


Fig 2: Statewise dengue cases

Epidemiological studies where dengue was identified[40]

Year of study	Areas of study	Type of DV detected
1964	VELLORE	DV 2
1966	VELLORE	DV 3
1968	VELLORE	DV 1,2,3,4
1968	KANPUR	DV 4
1969	KANPUR	DV 4, 2
1970	HARDOI	DV 2
1983	KOLKATA	DV3
1985	JALORE	DV 3
1988	DELHI	DV 2
1988	GUJARAT	DV 2
1990	KOLKATTA	DV 3
1993	MANGALORE	DV 2
1996	LUDHIANA	DV 1,2,3,4
1996	LUCKNOW	DV 2
1996	DELHI	DV 2
1996	DELHI	DV 2
1996	DELHI	DV2 – GENOTYPE IV

1996	**RURAL AREASOF HARYANA**	**DV 2**
1997	**DELH**	**DV 1**
2001	**DHARMAPURI, TN**	**DV 2**
2001	**GWALIOR**	**DV 2**
2001	**CHENNAI**	**DV 3**
2003	**DELHI & GWALIOR**	**DV 3**
2003	**KANYAKUMARI**	**DV 3**
2003	**DELHI**	**DV-3 (GENOTYPE III)**
2003-4	**DELHI**	**DV 3- SUBTYPE 3**
2003-5	**DELHI**	**2003 – DV 1,2,3,4** **2005 – DV 3**
2005	**KOLKATA**	**DV 3**
1956 - 2005	**ENTIRE COUNTRY**	**DV 2**
2006	**DELHI**	**DV 3**
2006	**DELHI**	**DV 1, 3**
2006	**DELHI**	**DV 1,3,4**
2006	**DELHI**	**DV-1, 2, 3 & 4**
2007	**MADURAI**	**DV 3(GENOTYPE 3)**
2007	**AP**	**DV 1, 4 (GENOTYPE I)**

2001-7	**DELHI & GWALIOR**	**DV 1- GENOTYPE III**
2008	**DELHI REGION**	**DV 1,2,3**
2008	**ERNAKULAM**	**DV 2,3**
2003-8	**DIFF PARTS OF COUNTRY**	**DV 3(GENOTYPE III)**
2007-9	**DELHI**	**DV 1,2,3,4**
2009-10	**Pune, Maharashtra**	**DV-4 (Genotype I)**

DENGUE VIRAL STRUCTURE

Dengue, the most prevalent arthropod-borne viral disease of humans, is caused by four serotypes of dengue virus (DENV 1-4).[41]

It is a febrile illness caused by dengue virus, a group of ss RNA virus belonging to the Flaviviridae family and Flavivirus genus. [42]

The genome is approximately 11 kb long and encodes a polyprotein precursor of about 3,400 amino acid residues. Co- and posttranslational processing by cellular and viral proteases generates three structural proteins, C (capsid) protein, M(membrane) protein, and E(envelope) protein, and seven nonstructural proteins, NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5.[43]

The viral particle consists of the RNA genome surrounded with C proteins and forms the inner core (Figure).

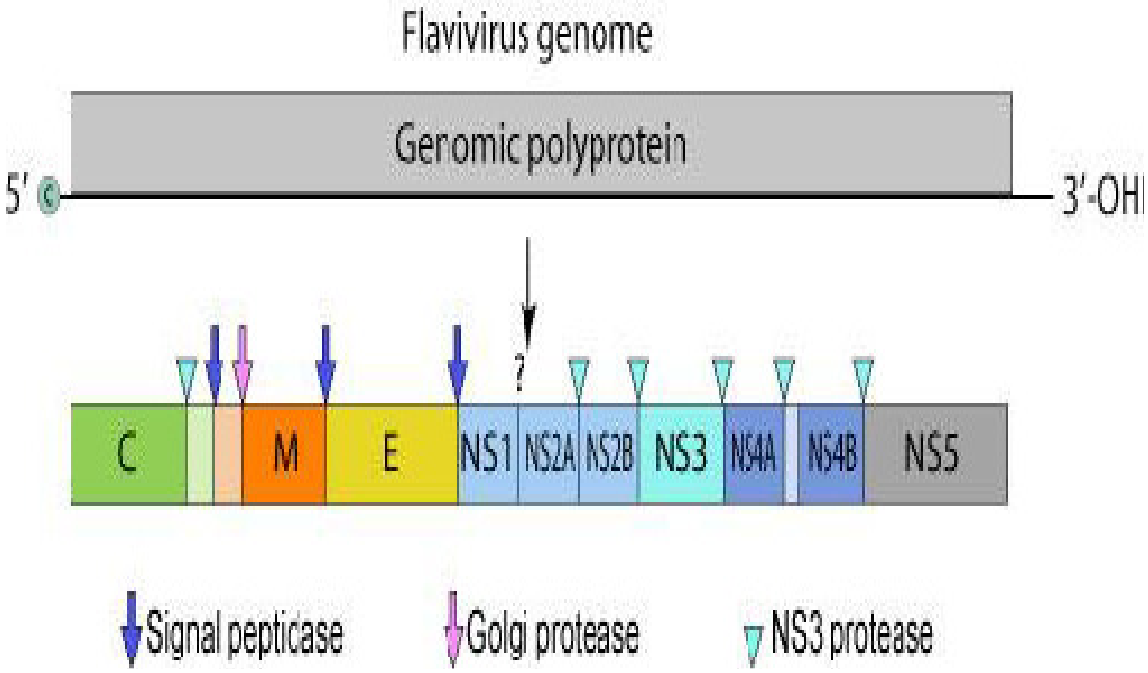


Fig 3: Schematic Representation of the Dengue Genome

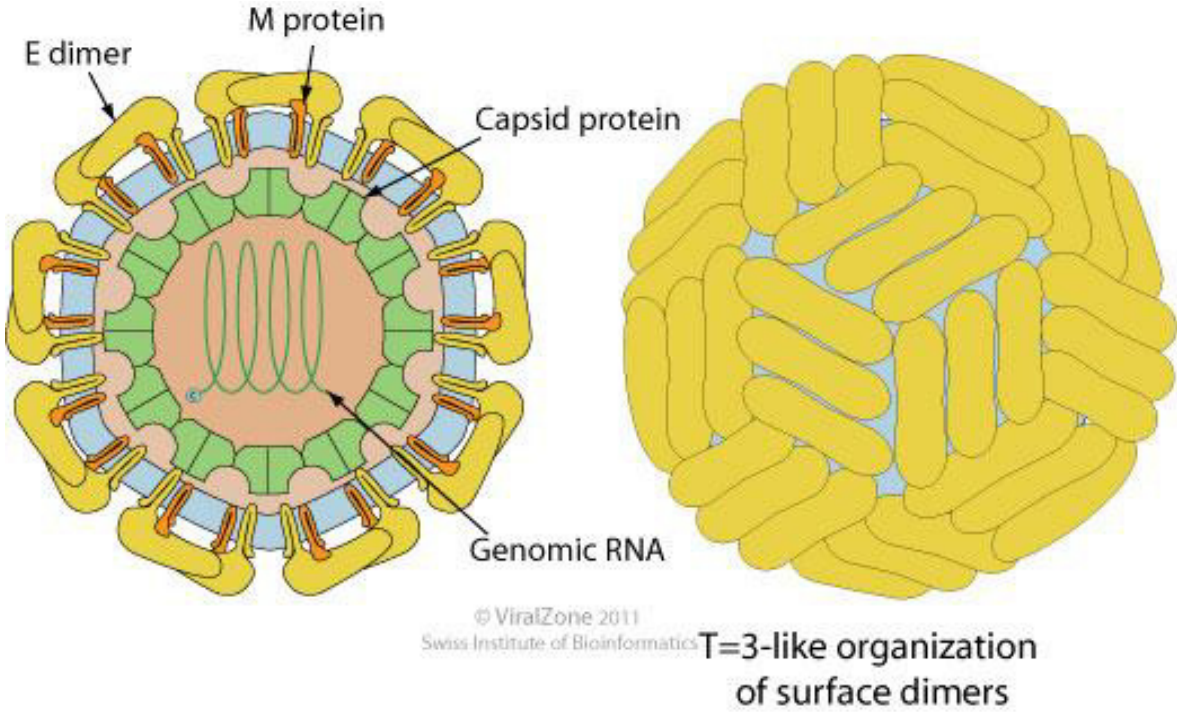


Fig 4: Dengue Envelope and Virion Structure.

The dengue spherical particle consists of the genomic RNA, surrounded by the capsid, then the envelope with E and M proteins bound.

The nucleocapsid is surrounded by a lipid bilayer in which M & E glycoproteins are inserted. In the infected cell the M protein is produced as precursor protein prM, which is believed to function as a chaperone during the folding and assembly of E protein.

The E glycoprotein are assembled as homodimers on the surface of mature virions and mediate the infectious entry of flaviviruses into cells. The crystal structure of the major external part of the E glycoprotein has been solved and reveals that the protein contains three distinct domains: domain I is the structurally central domain, domain II is the dimerization domain and contains fusion peptide, and domain III has an immunoglobulin like fold and mediates receptor binding.[44]

Dengue nonstructural glycoprotein-1 (NS1) is a glycoprotein of approximately 46 kDa in size. The functional role played by NS1 of dengue virus has not been found out. Besides its confirmed role in viral RNA replication, NS1 involvement in assembly and maturation of virus is not conclusive. [45]

NS2A (~22 kDa) is a hydrophobic protein generated by cleavage at the N-terminus by an unknown host signalase [46] and at the C-terminus by the viral protease and is thought to be membrane spanning. In addition a C-terminally truncated form of NS2A has also been reported in yellow fever virus to be important for infectious particle production. [47]

NS2B protein is an ER resident integral membrane protein of about 14 kDa and has been extensively studied as an essential cofactor for the activity of the NS3 serine protease. Its interaction with NS3 is important for the serine protease activity

and the cofactor activity is encoded in a conserved 40 residue hydrophilic region in the centre that is flanked by hydrophobic sequences which mediate membrane insertion.[48]

NS3 is a multifunctional protein of about 69 kDa. The N-terminal 185 aa residues include a serine catalytic triad which in complex with the NS2B cofactor functions as the viral protease that is required for polyprotein processing. [49]

Not much is known about the functions of the hydrophobic flavivirus proteins NS4A, and NS4B. It has been suggested that they contribute to the inhibition of the interferon-α/β (IFN-α/β) response. [50]

NS4A is a small (~16 kDa) hydrophobic protein and its C-terminus acts as a signal sequence for translocation of NS4B into the ER lumen.NS4B is an approximately 28 kDa hydrophobic integral membrane protein. Dengue NS4B protein localizes to cytoplasmic foci originating from the endoplasmic reticulum. [51]

Mutational analysis show that dengue NS4B could be involved in maintaining the balance between efficient replication in the mosquito vector and the human host.[52]

Thus far, the small hydrophobic protein NS4A (_16 kDa) is only poorly characterized, and its proper function for the viral replication cycle is still unknown. The observations that Kunjin virus (KUNV) NS4A localizes to the presumed sites of RNA replication and polyprotein processing and that an interaction between NS4A and NS1 is required for RNA replication suggests that NS4A is required in some steps of viral RNA replication.[53]

NS5 is a large (~103 kDa) well conserved multifunctional protein involved in RNA replication. It contains N-terminal RNA cap-processing activity and a C-terminal

RNA dependent RNA polymerase (RdRp) activity. A recent study demonstrated that NS5 induces IL-8 secretion late in infection suggesting that nuclear translocation of NS5 might modulate the transcription of cytokines such as IL-8 to perhaps counter the anti viral effects of innate immunity. [54]

Vectors of Dengue Virus:

Dengue virus is transmitted by Aedes aegypti and occasionally by other species such as Aedes albopictus. Vector breeding sites are most commonly found in the intra- and peri-domestic environment, however, pre-imago stages have been found in public spaces, cemeteries, schools, hospitals, health centres and hotels. [55]

The principal vector *A. aegypti*is is a small black and white mosquito, with tropical and subtropical geographic distribution. Artificial or natural water containers (water storage containers, flower pots, discarded tires, plates under potted plants, cemetery vases, flower pots, buckets, tin cans, clogged rain gutters, ornamental fountains, drums, water bowls for pets, birdbaths, etc.) that are within or close to places where humans live are ideally larval habitats for this mosquito.[56]

Aedes aegypti bites primarily during the day. This species is most active for approximately two hours after sunrise and several hours before sunset, but it can bite at night in well lit areas.[57]

Aedes albopictusis a small, dark mosquito with a white dorsal stripe and banded legs. The Asian tiger mosquito lays its eggs on the inner sides of water-holding receptacles in urban suburban, and rural areas as well as in nearby edges of forested areas. Aedes albopictus is a very aggressive daytime biter. Its peak feeding times are during the early morning and late afternoon.[57]

Transmission from one infected human to a mosquito, which later becomes infective and transmits the virus to a second human (Figure 5).[58]

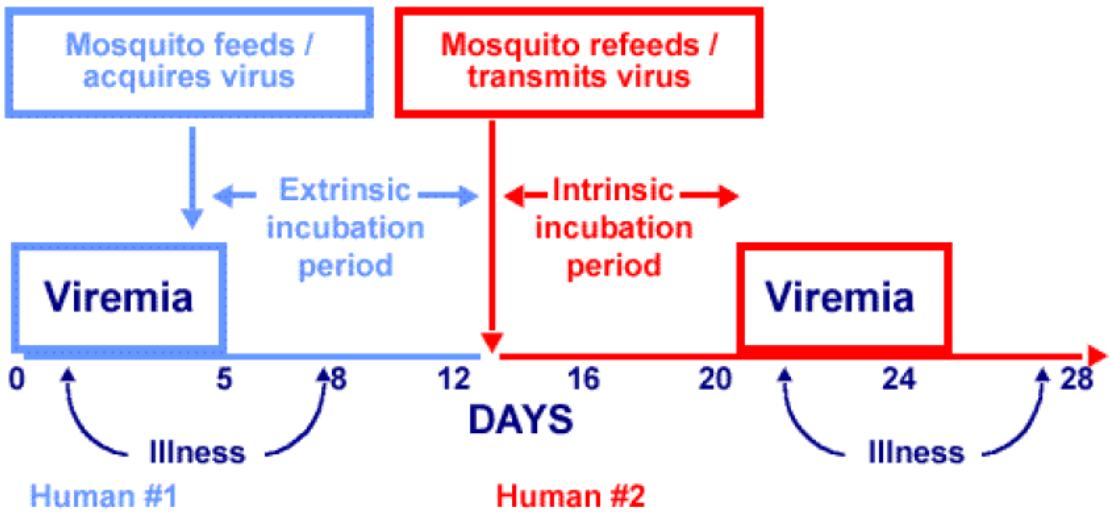


Fig 5 : Transmission cycle of DENV involving mosquitoes and susceptible hosts

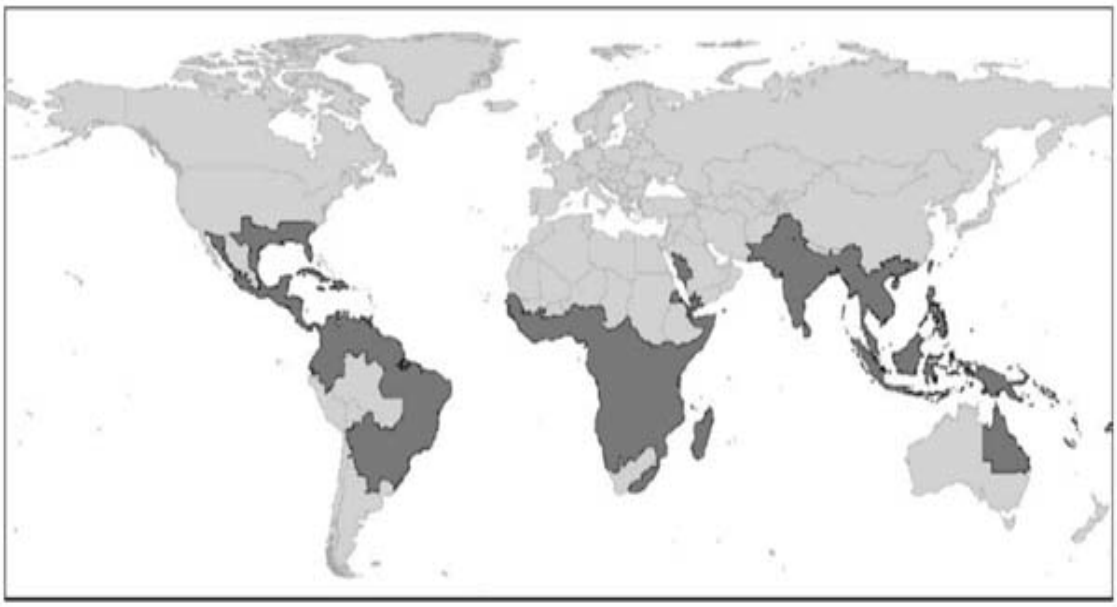


Fig 6 : Global distribution of Ae. Aegypti

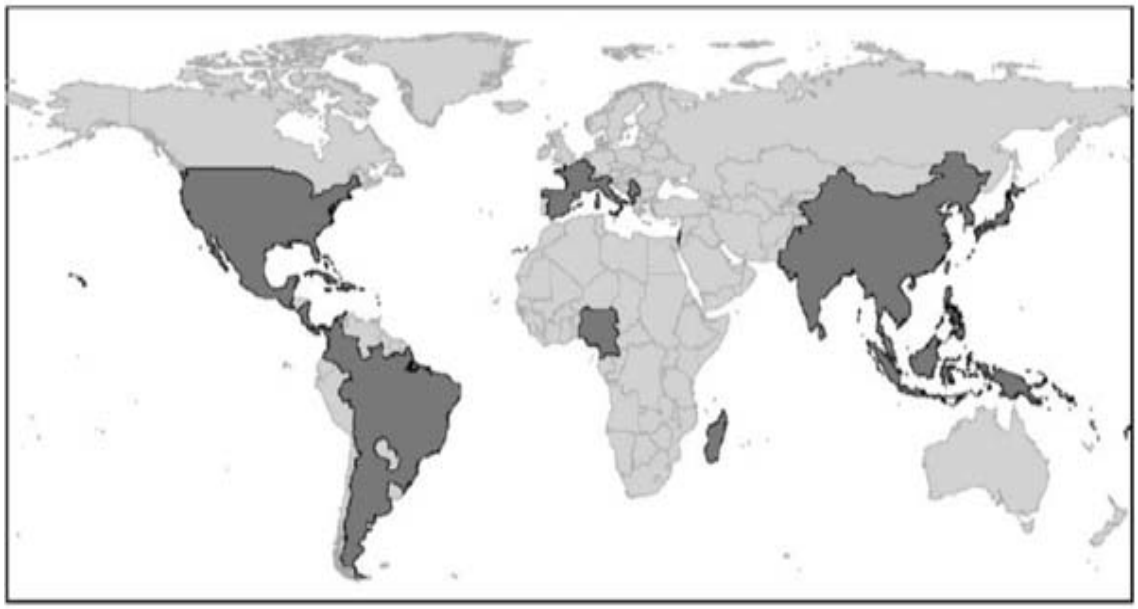


Fig 7 : Global distribution of Ae. albopictus

Dengue Viral Replication

DENV replication originates at the site of mosquito inoculation in resident cutaneous Langerhans dendritic cells (DCs), whose migration through the lymphatic system results in the induction of cytokines and the chemokine-mediated recruitment of immune cells.[59]

DV can infect different cell types, cells from the monocytic lineage, such as Langerhans cells in the skin and interstitial dendritic cells (DCs), are the primary viral targets. In these cells, expression of DC-specific intercellular adhesion molecule 3 (ICAM3)-grabbing non-integrin (DC-SIGN) is essential for productive DV infection.[60]

The findings that C-type lectin viral receptors, such as DC-SIGN or *N*-acetylgalactosamine-specific C-type lectin, are expressed by interstitial antigenpresentingcells (APC) beneath the epidermis of skin andmucosal tissue have underscored the role ofthese cells in viral dissemination and the antiviral immune response.[61]

After dengue virions are enclosed in endosomic vesicles they are exposed to acidic pH levels triggering structural modifications of the viral envelope E proteins. This conformational change leads to the exposure of a fusion peptide, which interacts with and bends the endosomic membrane towards the viral membrane, inducing the two membranes to fuse.[62]

Cellular lysosomal proteases within the endosomic vesicle uncoat the virion and digest the envelope, releasing the dengue genome into the ER. [63]

Nonstructural proteins, such as NS3 and NS4A, induce invaginations in the ER. Inside these invaginations RNA replication takes place. Membrane compartments separate viral RNAs and proteins from the cellular components allowing for efficient RNA synthesis and viral assembly. [64]

The ER facilitates the orientation of the capsid, allowing for the association between the viral RNA and the C protein to generate nucleocapsid complexes. [65]

This complex moves to the Golgi apparatus for post-translational modification with the addition of sugar residues. The complex buds through the ER lumen, with a prM-E-lipid envelope. While the virus is being exported via exocytic vesicles, mature M proteins form by furin-mediated cleavage of prM and causes E proteins to conformationally change to its homodimer form. Finally, progeny virus particles are released from the cell by exocytosis.[66]

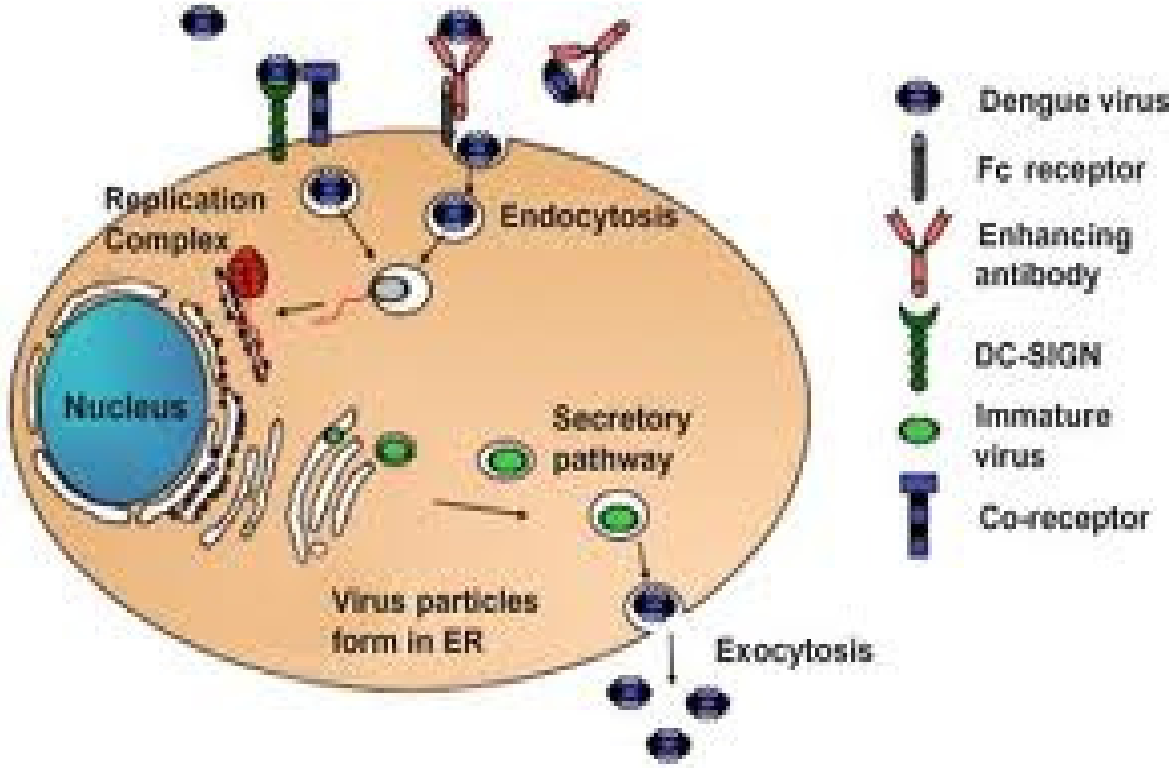


Fig 8: Dengue Replication Cycle.

Dengue enters a susceptible cell through receptor-mediated endocytosis. In endosomic vesicles, dengue virions are uncoated and release the genome into the ER. Viral RNA is translated into a polypeptide and processed to form viral proteins. Replication and viral assembly occurs in the ER, and the virions travel to the Golgi for modification and is exported via exocytic vesicles.

Pathogenesis of Dengue Infection

AFTER an infectious mosquito bite, virus replicates in lymph nodes and within 2-3days disseminates via blood to various tissues. It also replicates in skin, reactive spleen lymphoid cells, and macrophages.[67]

The malaise and flu like symptoms that typify dengueprobably reflect patients cytokine responsehowever myalgia a cardinal feature of illness indicate the

pathological changes in muscletypified by moderate perivascular mononuclear infiltrate with lipid accumulation.[68]

Histopathological examination of skin of patients with rash discloses minor degree of lymphocytic dermal vasculitis,and variably viral antigen. Viral antigen has been demonstrated in hepatocytes, kupffer cells and endothelia.[69]

Cell and tissue tropism of DENV may have a major impact on the outcome of DENV infections. In vitro data and autopsy studies suggest that three organ systems play an important role in the pathogenesis of DHF/DSS: the immune system, the liver, and endothelial cell (EC) linings of blood vessels.[70]

Cells of the immune system.

During the feeding of mosquitoes on humans, DENV is presumably injected into the bloodstream, with spillover in the epidermis and dermis, resulting in infection of immature Langerhans cells (epidermal dendritic cells [DC]) and keratinocytes. [71]

Infected cells then migrate from site of infection to lymph nodes, where monocytes and macrophages are recruited, which become targets of infection. Consequently, infection is amplified and virus is disseminated through the lymphatic system. As a result of this primary viremia, several cells of the mononuclear lineage,including blood-derived monocytes [72] myeloid DC and splenic and liver macrophages are infected. [73]

It should be noted that during secondary infections with heterologous DENV, high concentrations of DENV-specific immunoglobulin G (IgG) will complex newlyproduced virus that adheres to and is taken up by mononuclear cells.

Following infection, mononuclear cells predominantly die by apoptosis while abortively infected or bystander DC are stimulated to produce the bulk of mediators that are involved in inflammatory and hemostatic responses of the host.[74]

Organ pathology

The failure to isolate virus from most organ samples may indicate that those tissues contained primarily degraded virus or virus complexed with antibodies that prevent infection of cells in vitro.

The liver is commonly involved in DENV infections in humans and mouse models [75], with some reports suggesting an association between elevated liver enzyme levels and pontaneous bleeding tendencies Cases of dengue- associated hepatitis have been described, which were characterized by moderate midzonal hepatocyte necrosis, microvesicularsteatosis, and councilman bodies.[76]

Endothelial Cell(EC):

EC play an important role in the coagulation response upon severe systemic inflammation.[77]

DENV alters the endothelial cell surface protein production, its expression, and transcriptional activity. Expression of ICAM-1 (intercellular adhesion molecule-1) and beta integrin on micro vascular endothelium by DENV has been reported.. DENV also affects the expression of cytokine receptors. These may contribute to the mechanisms involved in plasma leakage in DHF. The role of DENV infected endothelial cells in the pathogenesis of coagulopathy in DHF is equally intriguing. There is upregulation of tissue plasminogen, thrombomodulin, protease activated receptor-1, and tissue factor receptor while there is downregulation of tissue factor inhibitor and activated protein C.[78]

Thrombocytopenia

Changes in platelet count by dengue virus-platelet interaction and the participation of anti-DV antibody to such changes were studied in vitro with the aim to investigate a mechanism and the results obtained showed that: (1) DV antigen attached to human platelets without immune-mediated reaction, (2) a decrease in platelet count was more markedly demonstrated by the binding of anti-DV antibody on the DV antigen associated with platelets than by the binding of the antigen-antibody complex on platelets, (3) a modulation of endothelial cell by the infection of DV to the cell was suggested as one of the causes of the thrombocytopenia.[79]

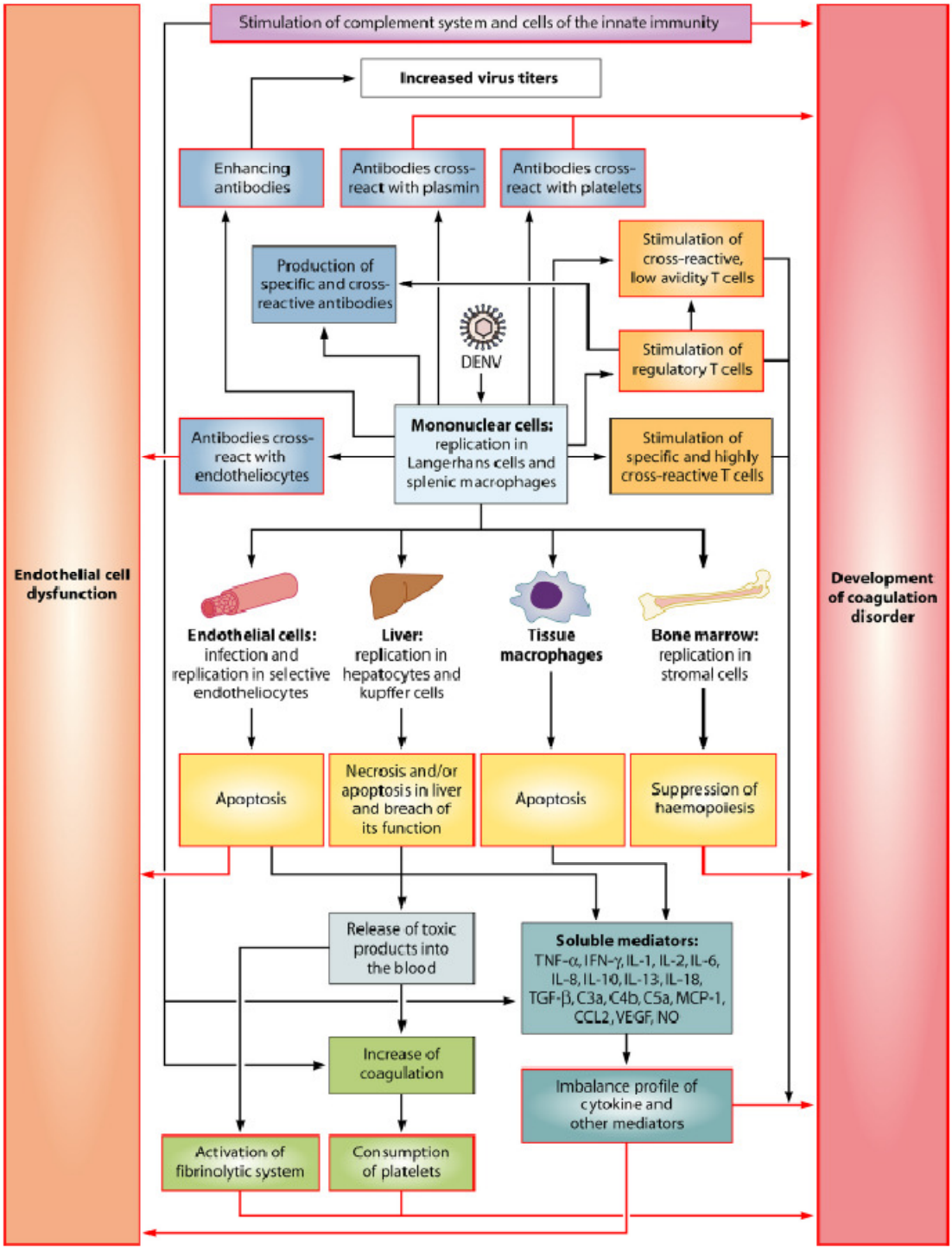


Fig 9: Pathogenesis of dengue virus

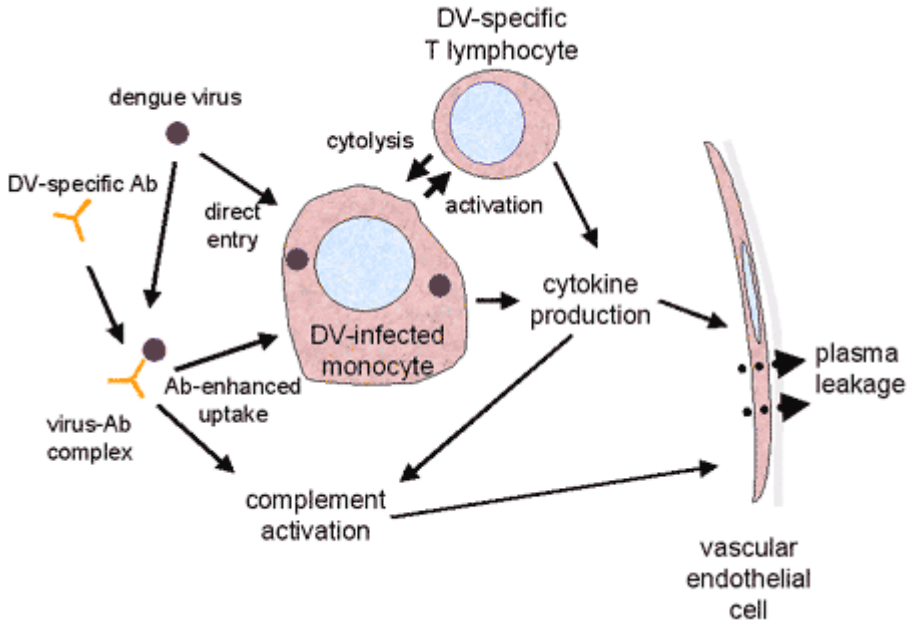


Fig 10:Immune response of dengue infection

Mechanism of dengue infection

Hypothesis	Mechanism	Result
ADE	Immune complexes	Enhancement of infection of target cells
T cell mediated	Cytokine production	Increased vascular permeability
Viral virulance	Highly virulent strains	Increased infection of target cells
Molecular mimicry	Autoimmune reactions	Haemorrhagic manifestations

CLINICAL DIAGNOSIS

Dengue virus infection may be asymptomatic or may cause undifferentiated febrile illness (viral syndrome), dengue fever (DF), or dengue haemorrhagic fever

(DHF) including dengue shock syndrome (DSS). The clinical manifestation depends on the virus strain and host factors such as age, immune status, etc.[80]

Infection with one dengue serotype gives lifelong immunity to that particular serotype, but there is only short-term cross-protection for the other serotypes.[81]

Manifestation of Dengue virus infections[80]

1. Asymptomatic
2. Symptomatic

A. Undifferentiated fever

B. (Classical) dengue fever
- Without haemorrhage
- With unusual haemorrhage

C. Dengue haemorrhagic fever(DHF)
- No shock
- Dengue shock syndrome(DSS)

Asymptomatic Infection:

As many as one half of all dengue infected individuals are asymptomatic, that is, they have no clinical signs or symptoms of disease.[82]

Undifferentiated Fever:

The first clinical course is a relatively benign scenario where the patient experiences fever with mild non-specific symptoms that can mimic any number of other acute febrile illnesses. They do not meet case definition criteria for DF.

The non-specific presentation of symptoms make positive diagnosis difficult based on physical exam and routine tests alone. For the majority of these patients, unless dengue diagnostic serological or molecular testing is performed, the diagnosis will remain unknown. These patients are typically young children or those experiencing their first infection, and they recover fully without need for hospital care.[82]

Dengue fever

Acute febrile illness of 2-7days duration (sometimes with two peaks) with 2 or more of the following manifestations:[83]

- Headache
- Retro-orbital pain
- Myalgia/arthralgia
- Rash
- Haemorrhagic manifestation
- Leukopenia

Classical dengue fever is characterized by acute onset of fever of 40- 41ºC, it may be accompanied by chills. This fever may last for 5-7 days followed by remission of few hours to few days, after which fever may appear again. Hence the name 'saddle back fever'. Fever is associated with the presence of severe frontal headache, retro-orbital pain, severe musculoskeletal pain, lumbar back pain, pain abdomen (break bone fever). There is also presence of anorexia, nausea, vomiting and hyperesthesia. Skin is initially flushed, but in 3-4 days with decrease in fever,

maculopapular rash appears which spares the palm and soles. Rash fades or desquamates, petichiae remain on the limbs. Following this fever appears again which is followed by recovery. Rarely, bleeding like epistaxis, gum bleeding, hematoma, gastrointestinal bleeding, hemoptysis may occur. This has to be differentiated from hemorrhage of DHS/DSS.Hepatitis is seen in some cases of DF with elevated liver enzyme levels. Central nervous symptoms have been reported occasionally with cerebral hemorrhage, edema, and rarely primary dengue encephalitis.[84]

Dengue haemorrhagic fever is characterised by appearance of haemorrhagic manifestations in addition to above while dengue shock syndrome is characterised by shock, capillary leakage and alered mental status.[85]DHF is a severe form of DF, caused due to infection with more than one denguevirus serotype. The first infection sensitizes the patient, and second appears toproduce an immunological catastrophe. Illness begins with sudden fever, headacheand facial flushing. This is associated with anorexia, vomiting, epigastric discomfort, right costal margin tenderness and general abdominal pain. Here, in the early periodsigns and symptoms are similar to undifferentiated fever. But in DHF there occurs plasma leakage and abnormal hematocrit, manifested by rising hematocrit andmoderate to marked thrombocytopenia.[86]

For purposes of description DHF is divided into three phases—namely:[5]

- Febrile,
- Critical (leakage), and
- Recovery (convalescent)phases

FEBRILE PHASE – lasts 2-7days

Hallmark features:

- high fever & symptoms consistent with either dengue fever

Complications:

- dehydration due to decreased fluid intake, emesis & increased metabolic rate
- Convulsions due to high fever
- Rarely haemorrhage

CRITICAL (leakage) PHASE – lasts 24-48 hrs

Hallmark features:

- Normal or subnormal temperature.
- Various degree of plasma leak into pleural or peritoneal spaces.
- Varying degrees of haemorrhage
- Risk of developing shock & death

Complications:

- Plasma leakage or haemorrhage leading to shock
- Intracranial bleed
- Metabolic abnormalities
- Coagulopathy
- Fulminant hepatic failure
- Shock leading to death

RECOVERY (convalescent)PHASE – lasts 2-4 days

Hallmark features:

- Resolution of plasma leakage & haemorrhage
- Stabilisation of vital signs
- Reabsorption of accumulated fluids
- Improved appetite & sense of well being

Complications:

- Intravascular fluid overload

Grading the severity of dengue infection: [86]

DF/DHF	GRADE	SYMPTOMS	LABORATORY
DF	-	FEVER WITH TWO OR MORE OF THE FOLLOWING SIGNS – headache, retro orbital pain, myalgia, arthralgia	Leucopenia, occasionally mild thrombocytopenia may be present. No plasma loss
DHF	I	Above signs plus positive Torniquet test	Thrombocytopenia≤ 1,00,000 /mm3, haematocrit≥20%
DHF	II	Above signs plus spontaneous bleeding.	Thrombocytopenia, ≤ 100,000, hematocrit rise ≥ 20%
DHF	III	Above signs plus circulatory failure, weak pulse, hypotension, restlessness.	Thrombocytopenia, ≤ 100,000 hematocrit rise ≥ 20%
DHF	IV	Profound shock with undetectable blood pressure and pulse	Thrombocytopenia, ≤ 100,000, hematocrit rise ≥ 20%

Grade III and IV are also called as DSS.

DENGUE SHOCK SYNDROME (DSS):

Early stages resemble DF/DHF, but in 2-7 days with defervecence of fever, there is reduced perfusion and early signs of shock are manifested with central cyanosis, restlessness, diaphoresis and cool, clammy skin and extremities. Initially, the blood pressure and pulse may be normal, but gradually there appears rapid and weak pulse, narrowing of pulse pressure to less than 20mm Hg, and in extremes unobtainable blood pressure. Platelet count declines, petichiae as well as spontaneous ecchymoses appear along with mucosal bleeding e.g. gastrointestinal bleeding. In 75% cases there is hepatomegaly with variable splenomegaly. Pleural effusion is seen in 80% cases. Adult respiratory distress syndrome may develop with capillary alveolar leak. If untreated, there occurs myocardial hypoperfusion and organ failure. In untreated cases, fatality rate is 50%, with proper treatment fatality is reduced to 1%.[86]

Usually dengue fever manifests as the above. Rarely, it may present atypically with unusual symptoms like: [87,88,89]

- Encephalopathy – due to hyponatremia, cerebral edema, microvascular frank hemorrhage. 0.5 to 6.2% patients with DHF develop this form dengue virus infection. Mortality rate is 22%.
- Cardiomyopathy or myocarditis.[90]
- Mononeuropathy, polyneuropathy, Guillain-Barré syndrome, transverse myelitis.
- Rhabdomyolysis
- Myalgia cruris epidemica – it is a rare manifestation and hemorrhagic shock is its rare complication. There are two mechanisms for this; direct invasion of the

muscle fibres and release myotoxic cytokines, particularly TNF. It is predominantly seen in boys of school going age group.[91]

- Liver failure
- Acute renal failure.

The new WHO classification for dengue severity is divided into Dengue without Warning Signs, Dengue with Warning Signs, and Severe Dengue.[82]

Dengue without warning signs:

Fever and two of the following –

- nausea, vomiting
- Rash
- Aches and pains
- Leucopenia
- Positive tourniquet test

Dengue with warning signs -

Dengue as defined above with any of the following:

- Abdominal pain or tenderness
- Persistent vomiting
- Clinical fluid accumulation (ascites, pleural effusion)
- Mucosal bleeding
- Lethargy, restlessness
- Liver enlargement >2 cm
- Laboratory: increase in HCT concurrent with rapid decrease in platelet count

Severe Dengue expanded

Dengue with at least one of the following criteria:

- Severe Plasma Leakage leading to:
    - Shock (DSS)
    - Fluid accumulation with respiratory distress
- Severe Bleeding as evaluated by clinician
- Severe organ involvement
    - Liver: AST or ALT $\geq$ 1000
    - CNS: impaired consciousness
    - Failure of heart and other organs

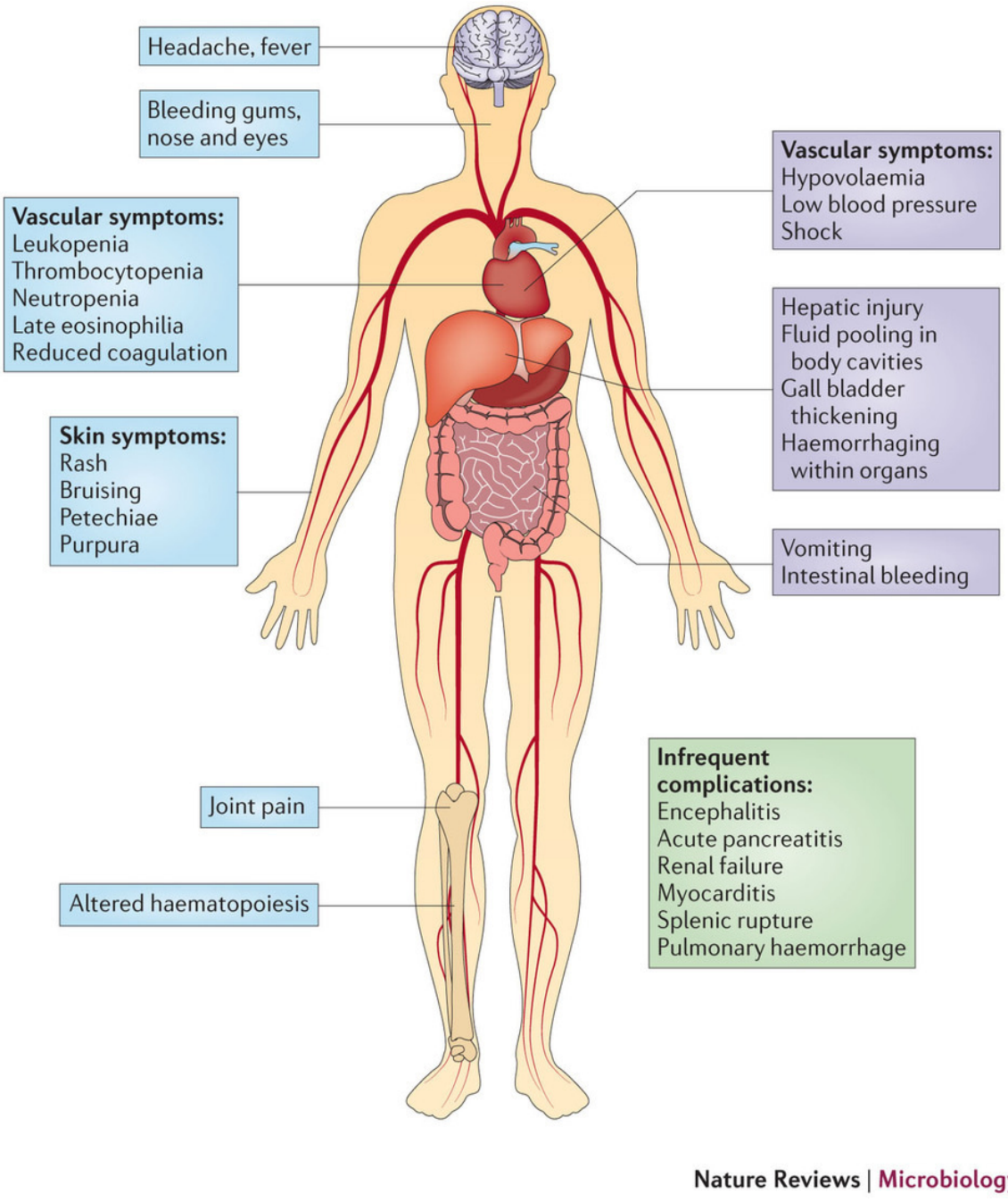


Fig 11: Clinical Presentation of Dengue Infection

Systemic infection with dengue virus (DENV) affects multiple organ systems. This diagram depicts the clinical symptoms and pathogenesis of dengue in humans, across the spectrum of mild to severe disease. Clinical manifestations associated with dengue fever are listed in blue boxes, those associated with dengue haemorrhagic fever are listed in purple boxes, and more rare complications of DENV infection are listed in the green box.

Differential diagnosis of dengue[92]

- **Arboviruses**

  Chikungunya virus (this has often been mistaken for dengue in South East Asia)

- **Viral diseases**

  Hantavirus; measles; rubella; enteroviruses; influenza; hepatitis A

- **Bacterial diseases**

  Meningococcaemia; scarlet fever; typhoid

- **Parasitic diseases**

  Leptospirosis; rickettsial diseases; malaria

Laboratory Diagnosis

Rapid and accurate dengue diagnosis is of paramount importance for:[92]

(i) Epidemiological surveillance;

(ii) Clinical management;

(iii) Research; and

(iv) Vaccine trials.

The following laboratory tests are available to diagnose dengue fever and DHF:

- Virus isolation – serotypic/genotypic characterization
- Viral nucleic acid detection
- Viral antigen detection
- Immunological response based tests – IgM and IgG antibody assays
- Analysis for haematological parameters

Specimens: Collection, storage and shipment[92]

Specimen type	Time of collection	Clot retraction	Storage	Shipment
Acute phase blood	0-5 days after onset	2-6hrs, 4˚C	Serum -70˚C	Dry ice
Convalescent phase blood	14-21 days after onset	2-24 hrs, ambient	Serum -20˚C	Frozen or ambient
Tissue	As soon as possible after death		70˚C or in formalin	Dry ice or ambient

Diagnostic methods for detection of dengue infection

During the early stages of the disease (up to six days of onset of illness), virus isolation, viral nucleicacid or antigen detection can be used to diagnose infection. At the end of the acute phase of infection, immunological tests are the methods of choice for diagnosis.

➢ Isolation of virus[93]

Isolation of dengue virus from clinical specimens is possible provided the sample is taken during thefirst six days of illness and processed without delay. Specimens that are suitable for virus isolationinclude:

1. Acute phase serum,
2. Plasma or washed buffy coat from the patient,
3. Autopsy tissues fromfatal cases (especially liver, spleen, lymph nodes and thymus),
4. Mosquitoes collected from theaffected areas.

A successful isolation of virus followed by serotyping usually takes more than two weeks which procrastinates the inevitable vector control under epidemic situations. Dengue viruses do not grow well in vitro normally.However, at present some more sensitive isolation systems comprising certain insect species such as nonblood sucking *Toxorhynchites* mosquitoes are available wherein the inoculation and isolation of dengue viruses are successfully accomplished.

(i) **Suckling mice:**

All the four dengue viruses have been successfully isolated in BS-C-1 cells (African green monkey kidney cells) or 1-3 days old baby mice using a soup prepared from Ae. Aegypti.[94] Baby mice are very insensitive before inculcating an evidenceof infection .In spite of this, suckling mice are important as it is generally not possible to detect the virus in other animal host body (e.g., mosquitoes,ticks) when in low quantity.

Mice is inoculated intracranially with classified suspensions of clinical specimens or macerated arthropod pools or animal tissues. Since the suckling mice is readily available in all laboratories and have certain practical advantages over others, the supernatant of the mosquito soup after centrifugation is inoculated intracerebrally into suckling mice for virus isolation.Dengue serotypes 1 and 4 were isolated from A.aegypti in 1961 from Vellore, in Tamil Nadu State, by inoculating infant mice.[95]

(ii) **Mosquito cell cultures:**

The choice depends on the availability of a host-cell cultures or mice that serve an indicator of virus infection, i.e., cytopathic effects in cell cultures, sign of illness or death in mice. This is the most common method for virus isolation. Thefirst Culex cell line in the country was established from the embryonic tissue of Cx. bitaeniorhynchus

mosquitoes. Cell lines from other two species of Culicine mosquitoes viz., Cx. infula, Cx. ambiguour, which are members of Cx.bitaeniorhynchus complex were also established subsequently.[93]

Recently, a new cell line from the embryonic tissue of Cx. tritaeniorhynchus mosquitoes was established. IFA technique is now routinely being used in the detection of virus antigen in infected cell lines and mosquitoes,Mosquito cell cultures particularly C6/36 (Ae. albopictus), AP-61 (Ae. pseudoscutellaris), Ae. krombeini, TR-248 (Toxorhynchites amboinensis), and other established mammalian cell culture lines (LLC-MK2 cells), is commonly methods for virus isolation.[96]

The C6/36 clone of A.albopictus cells is chosen for virus isolation because it demonstrated high sensitivity to denguevirus infection and ease of handling. These cell lines are highly stable and have optimal growth at lower temperatures than mammalian cells. The virus titre can be determined in all groups of mosquitoes using BHK-21 cells.For dengue virus isolation mosquito cell cultures have proved to be more sensitive than mice or mammalian cell culture systems.[97]

(iii) **Mosquito inoculation:**

Mosquito inoculation techniques are reported for detection and viral amplification [98]. This technique provided for the first time a sensitive method for isolation and assay of dengue viruses. Tx. splendens, a non haematophagous mosquito, was evaluated as a bioassay host for the detection and propagation of dengue viruses. All dengue virus serotypes and strains attained titres in Tx. splendens comparable to those observed for 2 strains of A.aegypti. .

The volume of inoculum tolerated by Tx. spendens is approximately 5 times greater than that injected into A.aegypti. The mosquito inoculation technique is used

for all titrations.[99] After incubation for 10 days at 32ºC individual mosquitoes are examined for the presence or absence of viral antigen in the salivary glands and brain tissue by the direct immunofluorescence antibody technique (IFA).Variations of the mosquito inoculation technique include inoculation of adult and larval Toxorhynchites mosquitoes.Isolation of viruses by intracerebral inoculation of the fourth instars of Tx. splendens larvae is routinely followed for dengue diagnosis.[100]

The virusesare detected by indirect immunofluorescence (IIFA) using a type-specific dengue monoclonal antibody.Mosquito inoculation techniques have been shown to be sensitive for isolation of flaviviruses.Inoculation of Tx. splendens larvae is relatively simple and safe and has been employed for isolation of dengue virus6.[93]

RECOMMENDED METHODS	**CONFIRMATION OF DENGUE FEVER INFECTION[80]**
➢ Inoculation of mosquitoes (*Aedesaegypti,Ae. albopictus, Toxorhynchitesamboinensis*and*Toxorhynchitessplendens*).	➢ Dengue virus generally replicates to hightitres (106 to 107 MID in an hour to fivedays). • Presence of antigens in head squashesdemonstrated by immunofluorescence (IFA) [Riman's test is the gold standard].
• Inoculation of insect cell cultures, namely C6/36, a clone of *Ae. albopictus*cells. ➢ Inoculation of mammalian cultures, Namely vero cells, LLCMK2 and BHK21.	➢ Presence of antigens in cells demonstrated by immunofluorescence (IFA). Viral titre is doneby RT-PCR. ➢ Cytopathic effect and plaque formation inmammalian cells – less efficient.

Cicelia Perret et al[101] - detected dengue virus for the first time in Chile, in an outbreak of dengue fever on Easter Island. The virus was isolated in tissue culture and characterized by reverse transcription–polymerase chain reaction as being dengue type 1.

Adrian Chappel et al[102]–conducted a study during the 1969 of dengue epidemic in Puerto Rico, where human sera and Aedes aegypti mosquitoes were collected for virus isolation and identification. Three methods of isolation were used and compared. In the first method, newborn mice was inoculated by the intracranial route, and was noted for any signs of illness, and serially passed specimens in mice until virus was isolated.

In the second method, tube cultures of LLC-MK2 cells were used, and any cytopathic effect (CPE) were noted, and assayed fluids for virus by plaque formation in LLC-MK2 cell monolayers.

In the third method original specimens were first inoculated into fluid cultures of Singh's A. albopictus cells. No significant CPE was seen in LLC-MK2 cultures; but distinct syncytial CPE was observed in A. albopictus cells. About the same number of virus isolates were made in each isolation system.

Virus isolates from both sera and mosquitoes were identified as dengue type 2 by a plaque-reduction neutralization test in LLC-MK2 cells.

Viral nucleic acid detection

Molecular techniques fulfil an important role in the diagnosis of dengue infection in early stages.[103]

Reverse transcriptase-polymerase chain reaction (RT-PCR)

This method is rapid, sensitive, simple, and if correctly standardised, it can be used for genome detection in human clinical samples, biopsies, autopsy tissues or mosquitoes.[104]

Chonticha et al[105], compared the results obtained by reverse transcriptase PCR (RT-PCR) with blood drawn into tubes containing EDTA and those obtained by RT-PCR with blood samples in serum separator tubes from 108 individualsclinically suspected of being infected with dengue virus. They determined that the extraction of RNA from whole blood followed by RT-PCR resulted in a higher detection rate than the use of serum or plasma. Using a selection of these samples, they also found that the ability to detect virus by direct C6/36 cell culture and mosquito inoculation was enhanced by using whole blood but not to the same extent as that seen by the use of RT-PCR.

Sergio et al[106] used five serological tests for the diagnosis of dengue infection: hemagglutination-inhibition (HI), complement fixation (CF), neutralization test (NT), immunoglobulin M (IgM) capture enzyme linked immunosorbent assay (MAC-ELISA) and indirect immunoglobulin G ELISA. Four methods of viral isolation are routinely used for dengue viruses: intracerebral inoculation of newborn mice, inoculation on mammalian cell cultures, intrathoracic inoculation of adult mosquitoes, and inoculation on mosquito cell cultures. Currently, dengue diagnosis is based on serology, viral isolation and RNA detection. Enzyme-linked immunosorbent assays (ELISA) are still the most widely used technique for serological diagnosis, but they do not identify the dengue virus serotype responsible for the current infection, so molecular techniques may soon assume a very important role in dengue diagnosis and hence they concluded that RT-PCR is definitely the most satisfactory test that can be

used on these infections, since it has been shown to be able to detect dengue viruses up to the 10th day after the onset of the symptoms.

Parag saxena et al [107]conducted study onfeasibility of multiplex reverse transcriptase PCR (M-RT-PCR) assay for clinical diagnosis and was validated with 620 acute phase dengue patient sera samples of recent epidemics in India. The comparative evaluation *vis a vis* conventional virus isolation revealed higher sensitivity. None of the forty healthy serum samples screened in the present study revealed any amplification, thereby establishing specificity of the reported assay for dengue virus only. And thus concluded that the findings clearly suggested that M-RT-PCR assay reported in the present study is the rapid and cost-effective method for simultaneous detection as well as typing of the dengue virus in acute phase patient serum samples.

Rajni kumaria and Anita chakravarti [108] conducted a study on reverse transcriptase–polymerase chain reaction (RT-PCR)–based rapid and cost-effective diagnostic test for detection as well as serotypic characterization of dengue viruses in the acute phase of illness. Serum samples of all individuals were evaluated by cell culture and RT-PCR. Of 200 clinically suspected patients tested, 66 were found to be positive for the dengue virus RNA and could be successfully characterized into the serotypes. Of these 66, 62 patients were found to be serologically positive by Dengue Duo IgM and IgG Rapid Strip test . The sensitivity of the cell culture procedure was found to be lower; 63% as compared with multiplex RT-PCR, with predictive value of positive test being 100% and predictive value of negative test being 84.8%. Specificity was found to be 100% for both the assays and thus proposed that 1-tube multiplex RT-PCR–based assay would prove to be an extremely useful tool for routine laboratory diagnosis.

B. Mishra et al [109]developed dengue chikungunya multiplex reverse transcriptase-polymerase chain reaction (DCmRT-PCR) and validated for simultaneous detection of dengue and chikungunya viral infections and its utility in virus serotyping. Blood samples from 97 suspected dengue and chikungunya cases and 10 healthy controls were subjected to dengue and chikungunya conventional RT-PCR and DCmRT-PCR. Thirty-one of 97 samples were positive for dengue or chikungunya viral RNA by RT-PCR and DCmRT-PCR with 100% concordance. DCmRT-PCR products were cycle sequenced. Seven dengue virus strains were clustered within genotype III of DENV-3 and 4 within genotype III of DENV-1, whereas chikungunya sequences were clustered within the Central/East African genotype. DCmRT-PCR was found to be a potential rapid test for simultaneous detection of dengue and CHIKV in clinical samples along with dengue serotyping.

Nested RT-PCR[110]

Single step 1st round RT-PCR is set on 50 µL final volume containing 5 µL of RNA suspension, 5 µL of 10 × PCR buffer II, 1µL of 10 mM dNTPs, 3 µL of 25 mM $MgCl_2$, 1.25 µL of 10pmoL/µl of forward primer D1, 1.25 µL of 10pmoL/µL of reverse primer D2, 0.25 µL of 1M DTT, 0.10 µL of reverse transcriptase from avian myeloblastosis virus (AMV RT, 10 U/µL, Promega, Madison, WI, 0.25 µL of AmpliTaq DNA polymerase (5U/µL), and 32.9 µL of RNase free water was applied in Thermocycler. The RT step is performed at 42°C for 60 min It is followed by 35 cycles of thermo cycling; 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min, using Gene Amp PCR system 9600.

Nested PCR

5 μL of 10 × PCR buffer II, 4 μL of 10 mM dNTPs, 5 μL of 25 mM $MgCl_2$, 1.25 μL of 10pmoL/μL of forward primer D1, 1.25 μL of 10pmoL/μL of each reverse primer, DNA polymerase (5U/μL, Applied Biosystems), and 24.50 μL of RNase free water. The RT-PCR product (2 μL) is then diluted to 1:100 dilutions in RNase free water. Five micro litter of this dilution is added in 45 μL of nested PCR mixture.

The nested PCR step is performed for 25 cycles of thermo cycling; 94°C for 30 sec, 55°C for 1 min, and 72°C for 2 min, using Gene Amp PCR system 9600.

Gel Electrophoresis

PCR products of both RT-PCR and Nested PCR steps (9 μL of PCR product with 1 μL of gel loading buffer) are electrophoresis in agarose gel and stained with ethidium bromide. The common band of 511 base pairs (bp.) in size is observed in the RT-PCR step, while in nested PCR step specimen containing DENV-1, 2, 3 or 4 is identified as the DNA band of 482, 119, 290, or 392 bp.[110]

De Paula S O [106]et al conducted study on nested-PCR which was developed followed by restriction enzyme (Kpn I) digestion of the amplicons to differentiate dengue-1 from dengue-2. Seventy-five IgM-containing samples were collected from 2 to 17 days after the beginning of the symptoms were examined. These samples were submitted to nested-PCR amplification, and virus was isolated from 2 (2.6%), 17 (22.7%) were positive by the regular PCR protocol, and 58 (77.3%) were positive by nested-PCR. All of the amplicons digested by Kpn I identified dengue-1 virus as the infecting strain. These results indicated that the nested-PCR provided a high yield of dengue genome amplification even in the presence of IgM antibodies, and restriction

enzyme digestion defined rapidly the circulating serotype. Therefore,they concluded that the combination of these techniques may be useful to rapidly identify dengue viruses in countries where dengue-1 and dengue-2 circulates, and this approach can also be applied to the other two serotypes.

Gomes et al [111]conducted study on, novel single-tube nested PCR (STNPCR) format, which was less prone to cross-contamination and reduces reaction cost and time. When standards for each dengue serotype were tested, the detection limit of the STNPCR was at least 10 copies for DENV-1 and 100 copies for DENV-2 and DENV-3, whereas the detection limit for the two-step RT-NPCR was 100 copies for each serotype. Sera from 22 patients with confirmed DENV-3 infections and from 14 healthy individuals were then tested in the STNPCR and the results indicated a sensitivity of 75.9% (CI 95%, 60.3–91.4) and a specificity of 100% for the RT-STNPCR. Thus their study concluded that RT-STNPCR was less sensitive than the conventional two-step RT-NPCR for the detection of virus in serum samples, but it was still adequately sensitive, and the advantages associated with a single-tube format outweigh the somewhat lower assay sensitivity, making it useful for diagnosis in the field.

Real-time RT-PCR

The real-time RT-PCR assay is a one-step assay system using primer pairs and probes that are specific to each dengue serotype.

The use of a fluorescent probe enables the detection of the reaction products in real time without need for electrophoresis. Manyreal-time RT-PCR assays have been developed either as ‘singleplex’ (only detecting one serotype at a time) or ‘multiplex’ (able to identify all four serotypes from a single sample). The multiplex assays have

the advantage that a single reaction can be used todetermine all four serotypes without the potential for introduction of contamination during manipulation of the sample The fourplex real-time RT-PCR assays are often less sensitive than nested RT-PCR assay but are faster.[112]

Yee ling lai et al [113]studied virus detection methodology for detection of dengue virus in the early phase of the disease. PCR,targeting cDNA derived from viral RNA, had been used as a laboratory-based molecular tool for the detectionof Dengue virus. They reported the development and use of three real-time one-step reverse transcriptase PCR(RT-PCR) assays to detect dengue cases and serotype the virus involved. Validation of the assays with local clinical samples collected from 2004 to 2006 revealed that there was an 88% positive correlation between virus isolation and RT-PCR with regard to dengue virus detection and a 100% correlation with seroconversion in subsequent samples. The serotyping results derived from duplex and fourplex assays agreed fully with each other and with that derived from immunofluorescence assays.

Gurukumar et al[114] sequenced 3'UTR of thirteen Indian strains of DENV and aligned with 41 representative sequences from GenBank. A region conserved in all four serotypes was used to target primers and probes for the qRT-PCR. The sensitivity of the two step qRT-PCR assay was10 copies of RNA molecules per reaction. The specificity and sensitivity of the assay was 100% when tested with a panel of 39 known positive and negative samples. Viral RNA could be detected and quantitated in infected mouse brain, cell cultures, mosquitoes and clinical samples. Viral RNA could be detected in patients even after seroconversion till 10 days post onset of infection. There was no signal with Japanese Encephalitis (JE), West Nile (WN), Chikungunya

(CHK) viruses or with Leptospira, *Plasmodium vivax*, *Plasmodium falciparum* and Rickettsia positive clinical samples. And thus concluded that the assay will be a useful tool for differential diagnosis of dengue fever in a situation where a number of other clinically indistinguishable infectious diseases like malaria, Chikungunya, rickettsia and leptospira occur.

Catherine L.K et al[115] developed a semi-nested polymerase chain reaction (PCR) assay based on primers within the NS3 gene for the simultaneous detection and typing of dengue viruses in human sera.A retrospective study was performed on acute sera from thirteen patients with dengue (confirmed by virus isolation) employing semi-nested PCR in parallel with virus re-isolation and a single-step RT-PCR method for the typing of dengue viruses in human sera.The semi-nested PCR assay could detect up to 1 pfu of dengue virus, but not other flaviviruses. The semi-nested PCR and single-step RT-PCR assays correctly typed dengue viruses in twelve and five sera, respectively, whereas none of the sera was positive by virus re-isolation. Thus the study concluded that semi-nested PCR assay is a sensitive and specific tool for the detection and typing of dengue viruses from viremic human sera.

Abdul Rahman et al [116]study aimed to develop rapid detection and serotyping methods for dengue virus using RT-PCR 2 primers (Dcon and peM). The whole blood samples were patients that had been confirmed with NS1 detection kit. The PCR products showed that in 12 samples, 100% were positive with different pattern among the serotypes especially for DEN1 and DEN2, but not for DEN3 and DEN4. This method was also able to confirm the double infection DEN2-DEN3, but not for the other ones because of the unspecific pattern. It is indicated that the RT-PCR 2 primers was a promising method for early detection and serotyping dengue virus infection.

Isothermal amplification method

The NASBA (nucleic acid sequence-based amplification) assay is an isothermal RNA-specific amplification assay that does not require thermal cycling instrumentation.[92]

Viral antigen detection

NS1 is a highly conserved glycoprotein which appears essential for viral replication, although no precise function has been assigned to it. Detection of dengue NS1 antigen indicate early dengue infection.[14]

NS1 is also a complement-fixing antigen and it produces a very strong humoral response. Because this protein is secreted, many studies have been dedicated to the utility of NS1as a tool for the diagnosis of infection with dengue virus. These studies focus on various aspects of diagnosis, including antigen-capture enzyme-linked immunosorbent assay (ELISA), and NS1-specific IgM and IgG responses.[117]

However recent studies that used ELISA and dot blot assays directed to the E/M antigen and the NS1 antigen demonstrated that high concentration of E/M and NS1 antigens could be detected in acute phase sera of both patients with primary and secondary dengue virus infections upto 9 days after onset of illness.[118]

Commercial kits for the detection of NS1 antigen in serum samples are available. These assays do not differentiate between the serotypes. As NS1 antigen appears early in infection and before the appearance of antibody, such assays are useful for early case detection and for outbreak investigations. Evaluations of these assays should be performed to assess their utility and cost–effectiveness.[117]

Ns1 assay–

Is a solid phase enzyme linked immunosorbent assay (ELISA) based on the "Direct Sandwich" principle. The microwells are coated with Anti-Dengue NS1 antibodies with high reactivity for Dengue NS1 antigen. The samples are added in the wells followed by addition of enzyme conjugate (monoclonal anti-dengue NS1 antibodies linked to Horseradish peroxidase (HPRO)). A sandwich complex is formed in the well wherein dengue NS1 (from serum sample) is ‘’trapped’’ or ‘’sandwiched’’ between the antibody and antibody HRPO conjugate. Unbound conjugate is then washed off with wash buffer. The amount of bound peroxidase is proportional to the concentration of dengue NS1 antigen present in the sample. Upon addition of the substrate buffer and chromogen, a blue colour develops. The intensity of developed blue colour is proportional to the concentration of dengue NS1 antigen in sample. To limit the enzyme-substrate reaction, stop solution is added and a yellow colour develops which is finally read at 450 nm spectrophotometrically.. Sample results are expressed in terms of index value. According to the manufacturer's recommendations, samples are considered (i) nonreactive (ii) equivocal for dengue virus NS1 Ag, and (iii) reactive for dengue virus NS1 Ag depending on the index value.[119]

Data S et al [119]studied on evaluation of the efficacy of NS1 antigen (Ag) assay as an early marker for dengue virus (DV) infection where they evaluated the performance of NS1 antigen (Ag) assay in comparison to MAC-ELISA, Six hundred acute/early convalescent sera were screened by both the assays. The detection rate increased to 320 (53.3%) when both the assays were used together on a single sample. NS1 Ag positivity varied from 71.42% to 28.4% in acute and early convalescent sera, conversely IgM detection rate was 93.61% and 6.38% in early convalescent and acute phase sera respectively ($P < 0.0001$). Thus the study concluded that NS1 Ag assay

holds promise in early diagnosis of dengue infection. When used in combination with MAC-ELISA on a single sample it significantly improved the diagnostic algorithm without the requirement of paired sera.

Kassim F M et al[120] - results revealed thatl the detection rate of dengue virus infection was similar for PCR and dengue antibody (65.9%) and for NS1 antigen and dengue antibody (62.0%) combinations. Therefore, the dengue NS1 antigen test can be used to complement the current antibody test used in peripheral laboratories. Thus, the combination of the NS1 antigen and antibody tests could increase the diagnostic efficiency for early diagnosis of dengue infection.

Pei yun shu et al[121] - used the dengue virus NS1 antigen (Ag) rapid test for on-site detection of imported dengue cases at airports. Among 22 positive cases of dengue identified from 850 patients with a fever suspected to have dengue, 17 were NS1 Ag test positive. Thus findings demonstrated the usefulness of the NS1 Ag rapid test in screening imported dengue cases at airports.

Mini Pritam Singh et al[122]compared IgM antibody detection with NS1 antigen for the diagnosis of acute dengue in 87 samples. NS1 antigen could be detected with good sensitivity (71–100%) till day 3 of fever, whereas IgM had a sensitivity of 0% to 50% at this time. On day 4 of illness, both the tests had comparative sensitivity. Beyond day 4, IgM antibody detection was superior to NS1. Both these diagnostic modalities were also compared with RT-PCR in 40 acute samples. NS1 detected additional 15 samples, which were missed by PCR. Hence concluded that NS1 antigen is an early diagnostic marker that is feasible in a routine diagnostic laboratory.

Serological tests

- Haemagglutination-inhibition (HI),
- Complement fixation (CF),
- Neutralization test (NT),
- NS1 ELISA
- IgM ELISA
- IgG ELISA.

Haemagglutination inhibition test(HI)

It is sensitive and easy to perform, requires only minimal equipment, and is very reliable if properly done. Because HI antibodies persist for long periods (up to 50 years or longer), the test is ideal for sero-epidemiologic studies.[92]

The HI test is based on the fact that the dengue viruses, under controlled conditions of pH and temperature, can agglutinate goose red blood cells, and this effect can be inhibited by specific antibodies. The antigens employed are prepared from infected suckling mice brains by extraction with sucrose and acetone to remove the lipids, or from infected mosquito cell cultures that have been concentrated or purified. Serum specimens must be treated to remove non-specific inhibitors and agglutinins.

The HI antibody usually begins to appear at detectable levels (titer of 10) by day five or six of illness, and antibody titers in convalescent-phase serum specimens are generally at or below 1:640 in primary infections, although there are exceptions. By contrast, there is an immediate anamnestic response in secondary and tertiary dengue infections, and antibody titers increase rapidly during the first few days of illness, often reaching 1:5,120 to 1:10,240 or more.

Thus, a titer of 1:1,280 or greater in an acute-phase serum is considered a presumptive diagnosis of current dengue infection. High levels of HI antibody may persist for 2-3 months in some patients, but in most antibody titers will generally begin to wane by 30-40 days and fall below the 1:1,280 level.

The major disadvantage of the HI test is lack of specificity, which makes the test unreliable for identifying the infecting virus serotype. However, some primary infections may show a relatively monotypic HI response that generally correlates with the virus isolated.[11]

Amin et al[123] conducted a hospital-based cross sectoral serological study in Chittagong metropolitan city – the second largest city in Bangladesh - to assess the current receptivity of the country for DF/DHF, using the haemagglutination inhibition test (HI). Samples were taken from the suspected cases of dengue infection following the inclusion and exclusion criteria. A total of 253 paired samples were collected from selected children aged between 1-15 years. A total of 18 (7.1%) samples were interpreted as positive ones out of which 9 (50.0%) were male children and the remaining 9 female children. Primary dengue infection was serologically diagnosed in 4 patients. Seven samples (male 4, female 3) produced results which were suggestive of definite secondary dengue infection while 1 male sample was interpreted as either primary or secondary dengue infection, and 6 (3 male, 3 female) samples were interpreted as presumedsecondary infection. Five to 9.9-year-old children were the most vulnerable group as 10 (55.6%) out of a total of 18 positive samples came from this group. Thus the study concluded that there is no room for complacency as regards to dengue infection.

Anantapreecha et al[124] conducted a study on 101 confirmed primary dengue cases. 48 were infected with type1, 10with type 2, 42 with type3, 1 with type4. HI titres were found to be same in all. And thus indicated that HI antibodies to dengue virus are cross reactive. Therefore while using HI test the cross reactivity should be considered.

Complement fixation test

The complement fixation or CF test is not widely used for routine dengue diagnostic serology.

The CF test is based on the principle that the complement is consumed during antigen-antibody reactions. Two reactions are involved, a test system and an indicator system. Antigens for the CF test are prepared in the same manner as those for the HI test.

CF antibodies generally appear later than HI antibodies, are more specific in primary infections, and usually persist for shorter periods, although low-level antibodies may persist in some persons. Because of the late appearance of CF antibodies, some patients may show a diagnostic rise by CF, but have only stable antibody titers by HI.

The greater specificity of CF test in primary infections is demonstrated by the monotypic CF responses, whereas HI responses are broadly heterotypic. The CF test is not specific in secondary infections. The CF test is useful for patients with current infections, but is of limited value for seroepidemiologic studies where detection of persistent antibodies is important.[92]

Pavri K M and Ghosh[125] confirmed the usefulness of CFT in isolation and identification of DEN V in human sera and mosquito suspensions as compared to neutralisation test.

Neutralization test

The neutralization test or NT is the most specific and sensitive serological test for dengue viruses used for determining the immune protection. The common protocol used in most dengue laboratories is the serum dilution plaque reduction neutralization test (PRNT).

PRNT is the most specific serological tool for the determination of dengue antibodies and is used to determine the infecting serotype in convalescent sera. This assay measures the titre of neutralizing antibodies in the serum of the infected individual and determines the level of protection the individual had against the infecting virus.

The assay is based on the principle of interaction of virus and antibody, resulting in inactivation of virus such that it is no longer able to infect and replicate in cell culture.

Some of the variability found in this assay is attributable to differences in interpretation of the results. The cell lines and virus seeds used as well as the dilution of the sera accounts for these differences.[97]

The microneutralization assay is based on the same principle as PRNT; however, instead of counting the number of plaques per well, this assay uses a colorimetric measurement of virus-induced cell lysis to determine the end-point dilution. This assay was designed to use small amounts of reagents and to be suitable

for the high-throughput testing of large numbers of samples. Some of the limitations of the assay include a poor correlation with PRNT results with samples from people with secondary infections.[117]

The Russell and Nisalak assay became known as the plaque reduction neutralization test (PRNT) and used prototype dengue seed viruses, monkey anti-sera controls, LLC-MK2 cell lines, and an agar overlaymedia with neutral red staining.[126]

Morens DM et al[97] studied on a newly modified semimicro plaque reduction neutralization test (PRNT) in BHK cells which was compared with a standard PRNT in bottles with LLC-MK2 monolayers and with an LLC-MK2 PRNT adapted to semimicro methods. The BHK semimicro PRNT compared favorably in terms of sensitivity in detecting dengue antibody (96%), specificity at a screening dilution (95%), and ability to detect seroconversion to dengue viruses of three serotypes (93%). The study concluded that the BHK PRNT was easier, faster, and more economical than either of the LLC-MK2 tests.

Matilu Mwau et al[127] conducted a study on seroprevalence of chikungunya, west nle virus and dengue virusvisiting health facilities wherein the positive samples by ELISA was confirmed by PRNT test. They collected 1578 serum samples from febrile adults with no evidence of malaria or typhoid exposure visiting Andersen Medical Clinic, Endebess Sub District Hospital and Kitale District Hospital and used indirect ELISAs to screen for exposure to Dengue, Chikungunya and West Nile Viruses, and Plaque Reduction Neutralisation Tests to confirm the status of those samples that were ELISA positive. Of the samples tested, 8.1% (127/1578) were CHIKV PRNT positive, 0.4% (6/1379) Dengue PRNT positive and 0.9% (12/1370) WNV Positive.

NS1 ELISA

The availibility of commercial ELISA assays to detect the DENV NS1 protein in acute plasma provides an additional dengue dianostic tool to the existing approaches of PCR, serology and less frequently, virus isolation.[128]

Kwoon Yong Poket al[129] studied the performance of three commercially available Dengue nonstructural 1 (NS1) antigen assays (Bio-Rad Platelia_ Dengue NS1 Antigen ELISA, PanBio DengueEarly ELISA, and Bio-Rad Dengue NS1 Antigen Strip test) and compared them with reverse-transcription polymerase chain reaction (RT-PCR) and other commercially available serological assays for the diagnosis of dengue.

The analysis showed RT-PCR to be the most sensitive and specific (100%) diagnostic method during the first 3 days of fever. The overall sensitivity of dengue NS1 antigen assays within the same period was 81.7%, indicating their potential role as a cost-effective and convenient alternative method to RT-PCR for the diagnosisof dengue fever in a primary healthcare setting.

Kumaraswamy et al[130] concluded that NS1 antigen capture ELISA gave an overall sensitivity rate of 93.3%. Sensitivity rate was significantly higher in acute primary dengue (97.4%) than in acute secondary dengue (68.8%).NS1 antigen capture ELISA KIT gave an overall sensitivity rate of 88.6% in the presence of anti dengue IgM and 96.3% in the absence of anti dengue IgM.

Chakravati et al [131]analyzed the utility of a commercial NS1 antigen based ELISA (Panbio Dengue Early ELISA) for detection of dengue infection during the early acute phase and anti-Dengue IgM capture ELISA for detecting dengue infection in patients in dengue endemic settings.

Of the 145 patient samples tested, 88 (60.7%) were positive for either NS1 antigen or IgM antibody by MAC ELISA. Dengue NS1 antigen-capture ELISA gave an overall positivity rate of 65.9% (58/88), and IgM ELISA gave an overall positivity rate of 60.2% (53/88). Only NS1 antigen was used to test during the first two days of fever. MAC ELISA showed positive by the third day of illness and gradually its positivity increased. From Day 3 to Day 7, no significant difference in detection rates was seen between the NS1 assay and MAC ELISA. Thus concluded that NS1 antigen assay may be a useful tool for detecting dengue infection during first few days of fever.

Chunya et al[132] established a new assay designated as "serotyping-NS1-ELISA" to detect the NS1 protein and to identify DENV serotypes simultaneously. When acute phase plasma from DENV-infected patients was used for validation, 65 out of 85 specimens (76.5% overall sensitivity) were positive to one of the four serotypes developed in assay. Identification of DENV serotypes by our NS1-ELISA was 100% accurate for DENV1, 3 and 4 and 82.4% for DENV2 as compared with standard RT-PCR. Assay specificity was 100% and thus concluded that the developed serotyping-NS1-ELISA provides an alternative for simultaneous detection of DENV NS1 and identification of its serotype in acute patients' specimens and the assay would be applicable for dengue diagnosis and epidemiological studies.

Shrivastava et al [133]evaluates a commercially available NS1 antigen capture ELISA vis-a-vis SD bioline Dengue NS1 antigen test for early detection of dengue virus. a total of 91 clinical samples were tested. Out of 91 samples, 24 (26%) were positive by NS1 antigen capture ELISA, 15 (16%) by SD bioline Dengue NS1 antigen test and 11(12%) positive by RT-PCR analysis. The RT-PCR-positive samples were further

subjected to virus isolation and resulted in three isolates. The results of the Panbio NS1 antigen capture ELISA, SD bioline Dengue NS1 antigen test, RT-PCR and virus isolation were correlated among themselves. Thus the study comprehensively established the utility of NS1 antigen ELISA in early diagnosis of dengue infection.

IgM-ELISA

Classic serological testing for dengue includes MAC-ELISA. This assay usesdengue-specific antigens from all four serotypes (DEN 1–4) for the capture of antidengue IgM-specific antibodies in serum samples. Most of the antigens used for thisassay are derived from the dengue virus envelope protein. The limitations of this testinclude the specificity of these antigens and cross-reactivity with other circulatingflaviviruses. These limitations have to be taken into account when working in regionswhere multiple flaviviruses co-circulate. IgM detection is not useful for the determinationof dengue serotypes owing to cross-reactivity of the antibody, even during primaryinfections.

MAC-ELISA is slightly less sensitive than the HI test for diagnosing dengue infection.

THE test is designed to detect IgM Antibodies to any one or three flaviruses prevalent in india.[134]

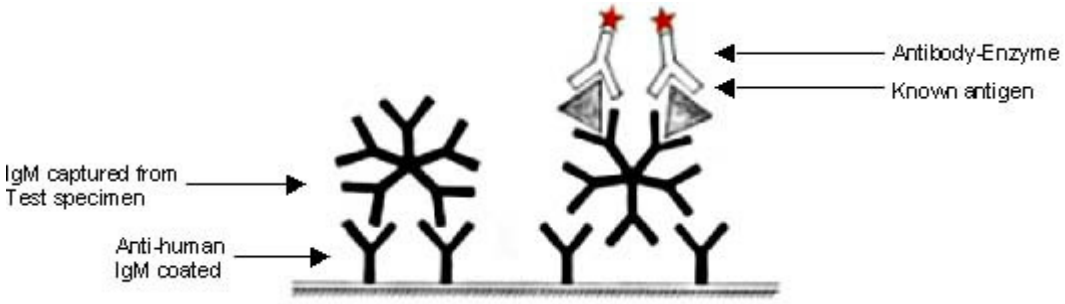


Fig 12: Principle of ELISA

PRINCIPLE OF THE TEST

It is a solid phase enzyme-linked immunosorbent assay (ELISA).

Plates are coated with goat IgG anti-human IgM (μ-chain-specific) antibody and blocked with BSA. Fifty μl of a 1/20 dilution of both the positive and the negative control serum samples and the test serum samples in PBS-0.5% BSA is added. After the plates are washed, 50 μl of a pool of antigens for the four dengue virus serotypes is added to each well at 16 hemagglutination units. After overnight incubation, 50 μl of a dilution of human anti-dengue virus IgG conjugate is added. *o*-Phenylenediamine and hydrogen peroxide in phosphate-buffered citrate (pH 5) are used as the substrate. The reaction is stopped, and the plates are read at 492 nm. The cutoff for the assay is two times the mean OD for the negative control serum sample.[135]

Satish et al [134]concluded in his study that NIV IgM capture ELISA (MAC-ELISA) showed a high positivity rate (38.9%) as compared to the PanBio Rapid (22.7%) and the PanBio IgM ELISA (20.7%). The NIV MAC-ELISA showed a high sensitivity (96%) as compared to PanBio Rapid (73%) and PanBio IgM ELISA (72%).

Satish et al concluded that MAC-ELISA though a 3 day procedure, would be a valuable screening test for the detection of IgM to dengue in routine diagnostic laboratories because of its high sensitivity and specificity rates. It also has the

advantage in that depending on the strength of the antibody units obtained to a specific flaviviral antigen, presumptive diagnosis as to which particular arboviral infection has occurred can be made in conjunction with clinical presentation and concluded that MAC ELISA would be a valuable screening test for the detection of IgM to dengue in routine diagnostic laboratories because of its high sensitivity and specificity rates.[54]

Carol et al [136]evaluated two new commercial dengue diagnostic tests, the MRL Diagnostics Dengue Fever Virus IgM Capture ELISA and the PanBio Rapid Immunochromatographic Test, on serum samples collected during a dengue epidemic in Jamaica. The MRL ELISA method correctly identified 98% (78 of 80) of the samples as dengue positive, while the PanBio test identified 100% (80 of 80). Both tests were 100% (20 samples of 20) specific indicating that both IgM ELISA and rapid tests provide excellent diagnostic tools.

IgG-ELISA : The classic IgG ELISA used for the detection of a past infection with dengue uses the same antigens as the MAC-ELISA. The assay is usually performed with multiple dilutions of the sera tested to determine an end-point dilution. This assay correlates with the haemagglutination assay used in the past. The higher the end-point dilution, the more robust the response obtained after the infection. In general, IgG ELISA lacks specificity within the flavivirus sero-complex groups;

Cardosa et al [137] conducted a study on convalescent sera from 69 individuals who were known to have had dengue or Japanese encephalitis virus infection were tested by western blotting against dengue, Japanese encephalitis and West Nile virus antigens. They determined that individuals who had been infected with dengue viruses had IgG responses against the premembrane protein of dengue viruses but not

Japanese encephalitis, whereas individuals who had been infected with Japanese encephalitis had IgG specific for the premembrane protein of Japanese encephalitis virus but not the dengue viruses. None reacted with the premembrane protein of West Nile virus. Using the Pearson Chi Square test, it was determined that the difference between the two groups was highly significant with a p value of <0.001.thus they concluded that the use of flavivirus premembrane protein in seroepidemiological studies will be useful in determining what flaviviruses have circulated in a community.

An excellent specificity of anti dengue-specific IgG(97%) was obtained by Baretto Dos Santos et al[138]. in an assay using a recombinant polypeptide located in the N-terminal portion of the envelope protein.

Although the detection of specific IgG has been superseded in the diagnosis of acute infection, seroepidemiological studies are best carried out using ELISAs to detect specific IgG. IgG avidity ELISAs can be used to determine whether an infection is primary or secondary, and can be more useful than the haemagglutination inhibition test for this purpose.

An indirect IgG-ELISA has been developed and compares well with the HI test. This test can also be used to differentiate primary and secondary dengue infections.

Flavia et al[139] developed an indirect enzyme-linked immunosorbent assay for detection of anti-dengue virus (DENV) immunoglobulin G antibodies using four recombinant DENV envelope polypeptides as antigens, which demonstrated a sensitivity of 89.4% and a specificity of 93.3% and these easily produced antigens were feasible, cost-effective alternative for generating reagents for dengue serological tests.

Barde P V et al [140]carried out a study to give reliable diagnosis of dengue infection in children and to detect circulating serotype in central India. Samples were collected from paediatric patients suspected to have dengue fever were subjected to IgM and IgG ELISA to determine dengue virus infection. Samples collected within 0-5 days of onset of illness and positive by IgM ELISA were tested by nested reverse transcription polymerase chain reaction (nRT-PCR). The PCR products were sequenced and analyzed. Of the 89 samples tested, 18 and 7 were positive for dengue IgM and IgG, respectively. They demonstrated dengue virus infection in children and adolescent in central India and concluded that it is important to monitor dengue virus activity at both serological and molecular level in this part of the country for better patient care and management.

Ganesh Oruganti et al[141] carried out a study on 200 apparently healthy individualsattending routine health check up. Samples were assayed for IgG antibodies by IgG capture ELISA and IgG indirect ELISA and confirmed using PRNT. Seropositivity of 10.5% with capture ELISA and 89.5% with indirect ELISA was seen and there was positive correlation between indirect ELISA and PRNT and concluded that IgG ELISA can be used to detect seropositivity among the individuals.

Interpretation of dengue diagnostic test[37]

HIGHLY SUGGESTIVE	CONFIRMED
One of the following	One of the following
• IgM +ve in a single serum sample	• RT-PCR +ve
• IgG +ve in a single serum sample with a HI titre of ≥ 1280	• Virus culture +ve
	• IgM seroconversion in paired sera
	• IgG seroconversion in paired sera or four fold IgG titre increase in paired sera

IgM/IgG ratio.

The IgM/IgG ratio is also used to distinguish primary from secondary infections with dengue. A dengue virus infection has been defined as primary if the capture IgM/IgG ratio is greater than 1.2, or as secondary if the ratio is less than 1.2.

Falconar et al.[142] compared dengue virus (DV) isolation rates and tested whether acute primary (P) and acute/probable acute secondary (S/PS) DV infections could be correctly classified serologically when the patients' first serum (S1) samples were obtained 1 to 3 days after the onset of symptoms (AOS). DV envelope/membrane protein-specific immunoglobulin M (IgM) capture and IgG capture enzyme-linked immunosorbent assay (ELISA) titrations ($1/\log_{10}$ 1.7 to 1 $\log_{10}$ 6.6 dilutions) were performed on 100 paired S1 and S2 samples from suspected DV infections. The serologically confirmed S/PS infections were divided into six subgroups based on their different IgM and IgG responses. Because of their much greater dynamic ranges,

IgG/IgM ELISA titer ratios were more accurate and reliable than IgM/IgG optical density (OD) ratios recorded at a single cutoff dilution for discriminating between P and S/PS infections. However, 62% of these patients' S1 samples were DV IgM and IgG titer negative (<OD_{max}/2 titer threshold), and in 35% of the S/PS infections, the patients' S1 and S2 samples were IgM titer negative. The IgM OD values were, however, much higher than those of IgG in the S1 samples of many of these, and the other, S/PS infections. This necessitated using higher (≥2.60 and <2.60) discriminatory gM/IgG OD (DOD) ratios on these S1 samples than those published previously to correctly classify the highest percentage of these P and S/PS infections. The DV isolation rate was highest (12/12; 100%) using IgG and IgM titer-negative S1 samples collected 1 day AOS, when 100% of them were correctly classified as P or S/PS infections using these higher DOD ratios.

Prince et al [143]conducted a study where they classified patients as having primary (*n* = 55) or secondary (*n* = 58) infections based on seroconversion patterns in a comparison of two sera collected <30 days apart and then evaluated IgM/IgG ratios and IgG avidity values(AVs) of the second specimens by using receiver operating characteristic curve analysis. The IgM/IgG ratio that best discriminated primary from secondary infection was 1.32; 95% of 55 primary infections exhibited ratios of >1.32, whereas 93% of 58 secondary infections exhibited ratios of ≤1.32. The discriminatory AV was 0.39; 95% of 41 primary infections exhibited AVs of ≤0.39, whereas 95% of 38 secondary infections exhibited AVs of >0.39. they also evaluated the IgM/IgG ratios and AV for primary-infection patients whose second serum samples were collected ≥30 days after the first serum samples; only 56% of 27 sera exhibited ratios of >1.32, whereas 81% of 21 sera exhibited AVs of ≤0.39, and thus concluded that

IgG AV is superior to the IgM/IgG ratio for distinguishing primary from secondary DV infections when using samples collected more than 5 weeks after disease onset.

Karyana Putu et al [144]conducted prospective study on children with suspected dengue hemorrhagic fever (DHF) who were tested for HI during acute and convalescent phase and the IgG and IgM titer were examined during the acute phase using ELISA method. Out of **s**ixty-two children, 48 were with secondary infection and 14 with primary infection. The prevalence of secondary infection was 77%. The best cut off point of the IgG to IgM ratio to predict secondary infection was >1.1 with sensitivity of 87.5%, specificity 92.9%, likelihood ratio 12.3, and post test probability 97.7%.and thus they concluded that IgG to IgM ratio of >1.1 is a good predictor for secondary infection.

Rapid diagnostic test (RDT)

A number of commercial rapid format serological test-kits for NS1 antigen, anti-dengue IgM and IgG antibodies have become available in the past few years, some of these producing results within 15 minutes.It is rapid, easily performed, interpreted early and has a extended shelf life.[145]

In an outbreak situation, if more than 50% of specimens test positive when rapid tests are used,dengue virus is then highly suggestive of being the cause of febrile outbreak.[92]

These kits were designed based on the principle that when a specimen is added to the sample well, anti-dengue IgG and IgM in the specimen will react with recombinant dengue virus envelope proteins-colloidal gold conjugates and forms a complex of antibodies-antigen. This complex will be captured by the relevant anti-human IgG

and/or anti-human IgM immobilized on the test device andgenerate a colored line when migrated along the length of the test device by capillary action. Similarly, dengue NS1 antigen captured by the anti-dengue NS1 Ag-colloid gold conjugate will migrate along the length of the device until being captured by the anti-dengue NS1 antigen immobilized on the membrane strips and generate a color line.[12]

Seok Mui Wang et al[12] evaluated a commercial Dengue Duo rapid test kit for early dengue diagnosis by detection of dengue virus NS1 antigen and immunoglobulin M (IgM)/IgG antibodies. A total of 420 patient serum samples were subjected to real-time reverse transcription-polymerase chain reaction (RT-PCR), in-house IgM capture enzyme-linked immunosorbentassay (ELISA), hemagglutination inhibition assay, and the SD Dengue Duo rapid test. Of the 320 dengue acute and convalescent sera, dengue infection was detected by either serology or RT-PCR in 300 samples (93.75%), as compared with 289 samples (90.31%) in the combined SD Duo NS1/IgM. The NS1 detection rate is inversely proportional, whereas the IgM detection rate is directly proportional to the presence of IgG antibodies. The sensitivity and specificity in diagnosing acute dengue infection in the SD Duo NS1/IgM were 88.65% and 98.75%, respectively. The assay was sensitive and highly specific and concluded that detection of both NS1 and IgM by SD Duo gave comparable detection rate by either serology or RT-PCR.

Subhash et al[146] conducted a study during outbreak in Delhi in 2010. Blood samples were tested for Dengue NS1, IgM and IgG using the single-step immunochromatigrahic test. Of the 1,886 patients screened, 678 and 1208 were NS1-positive and -negative respectively, in different combinations. In 394 cases, NS1 was exclusively positive while 29 were also IgG positive. In 942 cases NS1, IgM and IgG

were negative (triple negative).Testing for NS1 assisted the diagnosis of an additional 22.4% cases; of these 394 had evidence of primary infection and 29 of secondary infection. Thus 'Dengue Package' was useful in tackling the rise in suspected cases.

Pramiladevi R, Kaivalya, Shriram Kora[147] compared commercially available rapid immunochromatographic card test with IgM capture ELISA as gold standard. Probable dengue cases were diagnosed as per the WHO criteria and rapid immunochromatographic card test and IgM capture ELISA were conducted on the same serum sample.A total of 66 probable dengue cases were selected. 16 cases were found to be positive for dengue rapid immunochromatographic test, whereas 14 cases were found to be dengue positive by IgM capture ELISA. The study showed that the sensitivity of rapid card test is less(68.5%) but has a good specificity(86%). And concluded that in situations of epidemic, the card test can be used for screening but with the support of IgM capture ELISA.

Jayasimha et al[148] conducted study on seroprevalence and comparison of rapid test with Elisa for dengue NS1 antigen IgM and IgG. They tested 226 serum samples out of which150 were positive by ELISA.seroprevalence wsa 66%, RAPID TEST SHOwed sensitivity of 80% and efficiency of 87.16% and thus concluded that rapid test can be used as screening test for dengue infections.

DOT BLOT IMMUNOASSAY

Relatively new, and reagents and test procedures are evolving. At least one dot blot immunoassay for dengue antibodies is available commercially.[92]

Cuzzubo et al [149] compared Dengue IgM and IgG Dot Blot assays and the Dengue Duo IgM and IgG Capture ELISA. Sensitivity of Dot Blot IgM ELISA was

higher than the IgM ELISA (100 vs. 95%), while the IgM ELISA showed higher specificityin JE (100 vs. 20%) and non-flavivirus infections (100 vs. 97%). Defining elevation of either IgM or IgG as a positive result, the Dot Blot and ELISA tests both showed 100% sensitivity in dengue infection, while the elisa showed superior specificity in JE (70 vs. 0%) and non-flavivirus infections (100 vs. 67%). Both were useful aids to the serological diagnosis of dengue infection.

Falconer AKI, Romero-Vivas CME[150] generated mouse monoclonal antibodies and screened against NS1 glycoprotein purified from each Dengue serotype. One Mab, Mab 2C4.6, was further tested against these DEN V glycoproteins in human sera using simple peroxidase labelled secondary antibody/ substrate – developed dot blot assays. 2C4.6 Mab showed a sensitivity of less than 32 ng/ml for NS1 glycoprotein and thus they concluded that this was a simple, inexpensive robust assay and could be used as an ideal method for detection of DENV in endemic areas.

Sjahrurachman et al [151] conducted a study on semiquantitative dot immunoassay for detection of IgM-anti dengue antibodies in human sera employing biotinylated-dengue antigens which is simple in term of methodology and rapid in terms of the test result. Out of 146 paired sera, 23 showed primary dengue infection,48 showed acute secondary dengue infection, and 33 showed recent dengue infection and thus concluded that this test was more sensitive for diagnosis of secondary dengue infection as compared to HI test and the test result can be obtained within four and a half hours.

HAEMATOLOGICAL TESTS

Standard haematological parameters like platelet count, haematocrit are important and part of biological diagnosis of dengue infection.

Thrombocytopenia, a drop in platelet count below 100000/µl may be occasionally observed in dengue fever but is a constant feature in DHF. It is usually found between 3rd and 8th day of illness, often before or simultaneously with changes in haematocrit. Haemoconcentration with an increase in the haematocrit of 20% or more is considered to be a definitive evidence of increased vascular permeability and plasma leakage.[92]

Francisca raimunda et al[152]: conducted a study on correlation of different laboratory tests during the evolution of dengue fever, comparing frequencies between the different clinical forms in order to use test results to predict the severity of the disease. Thrombocytopenia and elevated transaminases were observed in patients with classic dengue fever. The main laboratory abnormalities found in dengue hemorrhagic fever were thrombocytopenia, hemoconcentration and elevated transaminases, similar to severe dengue with the exception of hemoconcentration.

Most laboratory abnormalities started on the 3rd day but were moreevident on the 5th day with restoration of values by the 11thday; this was more prominent in under 15-year-olds and with the more severe clinical forms and conclude that these results are relevant in assessing the disease because they can be used as markers for more severe forms and can help by enabling the adaptation of the therapeutic conduct to the needs of individual patients.

Abhinav Jain et al[153] conducted study on the clinical and haematological profile of Dengue outbreak and its cost impact. 56 patients from medical, dental and nursing fraternity with Dengue were studied. The most common abnormalities that predict severity were raised hematocrit of >40% and a low platelet count of <50,000/mm3.

Lab diagnosis of Dengue in India:

Diagnosis of DV infection is routinely done by demonstration of anti DV IgM antibodies or by NS-1 antigen in patients' serum depending upon day of illness using ELISA kits (prepared by National Institute of Virology, Pune) and commercial kits(rapid). [131]Molecular methods (reverse transcriptase PCR) are being increasingly used in diagnosis of DV infection. A single tube nested PCR for detection and serotyping of DV was developed and used for detection of co-infection by two viruses[109]. DV isolation in tissue culture cells and its sequencing is also being done.[154]

MANAGEMENT

There is no specific treatment for dengue other than supportive measures and judicious fluid therapy. The danger period of dengue or the so-called "critical period" where the patient might undergo sudden deterioration is relatively short and is between 48–72 hours. If the patient is appropriately managed during this phase, the chance of a fatal outcome is minimal.[155]

GUIDELINES FOR TREATMENT[80]

1. GROUP A – patients with uncomplicated disease who may be sent home
2. GROUP B - patients for in hospital management
3. GROUP C - patients who require emergency treatment and urgent referral

Dengue Case Management

GROUP A
GROUP CRITERIA: patients whodo not have waning signs and who are able 1. To tolerate adequate amount of fluids 2. Pass urine 3-4 times/day
LABORATORY TEST 1. Full Blood Count 2. Haematocrit
TREATMENT 1. Advice adequate bed rest 2. Adequate fluid intake 3. Paracetamol 4gram max per day Patients with stable haematocrit can be sent home
MONITORING 1. Daily review for warning signs 2. Advice to immediate return to hospital if any warning sign seen 3. Written advice of management

<table>
<tr><th colspan="2">GROUP B</th></tr>
<tr><td colspan="2">GROUP CRITERIA
Patients with any of the following criteria Existing warning signs OR co existing conditions (pregnancy, infancy, old age, diabetes, renal failure), living alone, far away from hospital</td></tr>
<tr><td colspan="2">LABORATORY TEST
1. Full Blood Count
2. Haematocrit</td></tr>
<tr><td>TREATMENT
Obtain reference Hct before fluid therapy
➢ Give isotonic solutions such as 0,95 saline, Ringer lactate, start with 5-7 ml/kg/hr for 1-2 hours, then reduce to 2-3 ml/kg/hr or less according to clinical response

Reassess clinical status and repeat Hct
➢ If Hct remains the same or rises only minimally -> continue with 2-3 ml/kg/hr for another 2-4 hours
➢ If worsening of vital signs and rapidly rising Hct -> increase rate to 5-10 ml/kg/hr for 1-2 hours.</td><td>TREATMENT
➢ Encouragement for oral fluids
➢ If not tolerated, start intravenous fluid therapy 0,9% saline or Ringer Lactate at maintenance rate</td></tr>
</table>

Reassess clinical status, repeat Hct and review fluid infusion rates accordingly ➢ Reduce intravenous fluids gradually when the rate of plasma leakage decreases towards the end of the critical phase. **This is indicated by:** ➢ Adequate urine output and/or fluid intake ➢ Hct deceases below the baseline value in a stable patient.	
MONITORING ➢ Vital signs and peripheral perfusion (1-4 hourly until patient is out of critical phase ➢ Urine output (4-6 hourly) ➢ Hct (before and after fluid replacement, then 6-12 hourly) ➢ Blood glucose (before fluid replacement and repeat as indicated ➢ o Other organ functions (renale profile, liver profile, coagulation profile, before fluid replacement and as indicated)	**MONITORING** ➢ Temperature pattern ➢ Volume of fluid intake and losses ➢ Urine output – volume and frequency ➢ Warning signs ➢ Hct, white blood cell and platelet counts

GROUP C

GROUP CRITERIA

Patients with any of the following features.

- Severe plasma leakage with shock and/or fluid accumulation with respiratory distress
- Severe bleeding
- Severe organ impairment

LABORATORY TESTS

- Full blood Count (FBC)
- Haematocrit (Hct)

TREATMENT OF SHOCK:

- Start intravenous fluid resuscitation with isotonic crystalloid solutions at 5-10 ml/kg/hr
- Reassess patients' s condition,

If patient improves:

- Intravenous fluids should be reduced gradually to 3- 5 ml/kg/hr, then to 2-3 ml/kg/hr and then depending on haemodynamic status
- Can be maintained for up to 24 - 48 hours

If patient still unstable:

- Check Hct after first bolus
- **If Hct increases/** still high (>50%), repeat a second bolus of crystalloid solution at 10-20 ml/kg/hr.
- If improvement after second bolus, reduce rate to 7- 10 ml/kg/hr, continue to reduce as above

- **If Hct decreases,** this indicates bleeding and need to cross-match and transfuse blood as soon as possible

TREATMENT OF HYPOTENSIVE SHOCK

- Initiate IV fluid resuscitation with crystalloid or colloid solution at 20 ml/kg as a bolus for 15 min

If patient improves

- Give a crystalloid / colloid solution of 10 ml/kg/hr, them reduce gradually

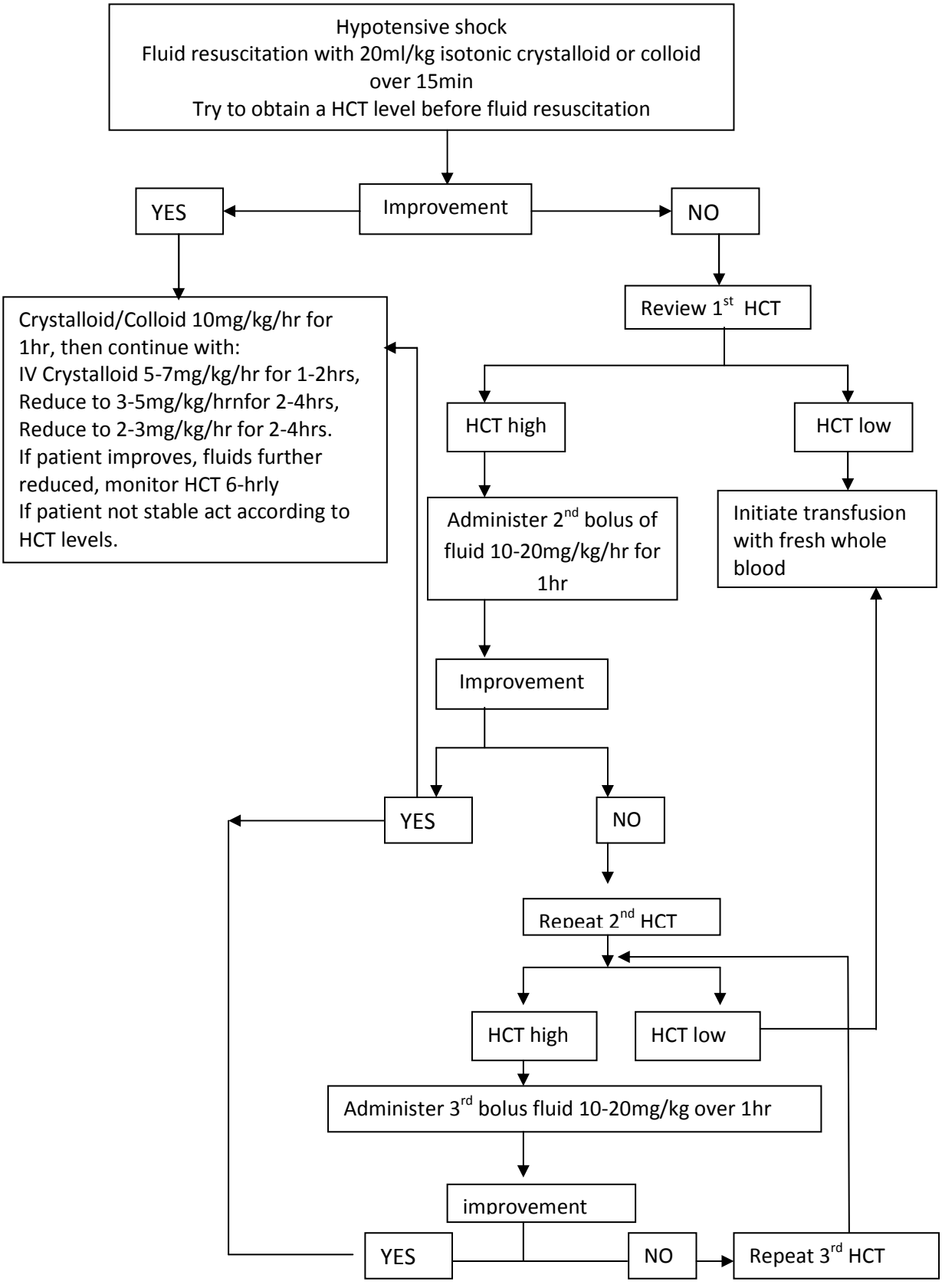
If patient still unstable

- Check Hct after the first bolus
- **If Hct increases/** still high (>50%), change IV fluids to colloid solutions at 10 ml/kg/hr, then reduce to 7- 10 ml/kg/h, then change back to crystalloid solution and reduce rate as above
- **If HCT decreases,** this indicates bleeding, see above

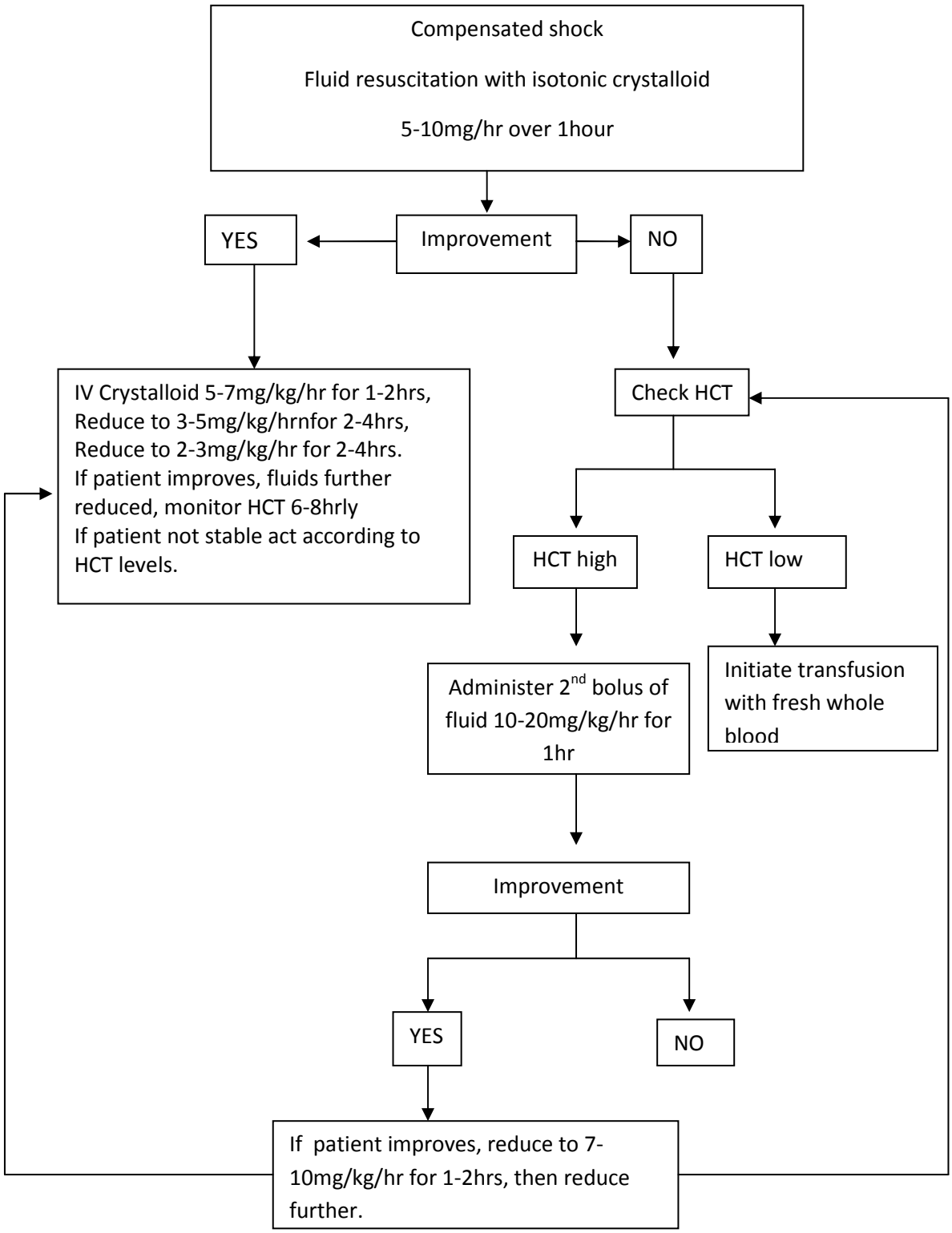
TREATMENT OF HAEMORRHAGIC COMPLICATIONS:

- Give 5-10 ml/kg of fresh packed red cells or 10-20 ml/kg of fresh whole blood

FLOW CHART-[156]



ALGORITHM OF HYPOTENSIVE SHOCK MANAGEMENT



ALGORITHM OF COMPENSATED SHOCK MANAGEMENT

ANTI VIRAL DRUGS

The preferable new treatment for dengue would be an antiviral drug. At present, a specific antiviral drug is not available; however, there have been a lot of attempts to discover one. In phytomedicine, several sulfated polysaccharides extracted from seaweeds have been studied and high antiviral activity against dengue virus has been observed.[157] In modern medicine, ribavirin, glycyrrhizin and 6-azauridine are reported to have cytostatic and inhibitory effects on the dengue virus.[158] An adenosine analog is another promising drug currently being studied. The chemical 'NITD008' is the best example.[159]

The challenge of developing dengue antivirals

Development
• Several potential viral targets, of which the most advanced are NS3/NS2B protease and NS5 polymerase; work in progress on E, NS3 helicase, and NS5 methyltransferase. • Must be active against all serotypes. • Must be effective in both primary and secondary DEN infections. • Must be active orally stable to heat and humidity, have a long shelf-life, and have low/reasonable production costs. • Exploration of cellular targets • Good safety profile, including few or no secondary effects. • Useful in infants, children and adults.

Implementation
• Need for rapid point-of-care diagnostic tool to apply antiviral most effectively. • Short window of viraemia. • Possible development of resistance: use cocktails of multiple drugs to avoid this eventuality. • Must be tested in acute DEN infection, and a prophylactic trial is not an option

PREVENTION AND CONTROL MEASURES:[160]

1. ENVIRONMENTAL MANAGEMENT
2. PERSONEL PROTECTION
3. BIOLOGICAL CONTROL
4. CHEMICAL CONTROL

ENVIRONMENTAL MANAGEMENT:

- **IMPROVED WATER SUPPLY – it is essential that potable water** supplies be delivered insufficient quantity, quality and consistency to reduce the necessity and use of water storage containers that serve as the most productive larval habitats.
- **MOSQUITO PROOFING OF OVERHEAD TANKS/ CISTERNS OR UNDERGROUND RESERVOIRS –** tanks, cisterns and masonary chambers should be water proofed. Similarly, mosquito proofing of domestic wells and underground water storage tanks should be undertaken. Masonary chambers of

sluice valves and water meters are required to be provided with soak pits as part of preventive maintainance.[161]

- **DRAINING OF WATER SUPPLY INSTALLATIONS**
- **DOMESTIC STORAGE – water storage** containers should be covered with tight fitting lidsor screens, care being taken to replace them after water is used.[162]
- **BUILDING EXTERIORS –** design of buildings is important to prevent Aedes breeding. Periodic inspection of building during rainy season to locate potential breeding sites.
- **SOLID WASTE DISPOSAL –** solid wates namely tins, bottles, buckets or any other waste material scattered around houses, should be removed and buried in land fills.household and garden utensils should be turned upside down to prevent water accumulation.
- **FILLING OF CAVITIES OF FENCES –** fences and fence posts made from hollow trees such as bamboo should be cut down to the node and concrete blocks should be filled with packed sand, crushed glass, or concrete to eliminate aedes larval habitats.[160]

PERSONEL PROTECTION:

- **PROTECTIVE CLOTHING –clo**thing reduces the risk of mosquito biting if the cloth is sufficiently thick or loosely fitting. Long sleeves and trousers with stockings may protect arms and legs, the preferred sites for mosquito biting.[160]
- **MATS, COILS AND AEROSOLS**
- **REPELLENTS –** classified into 2 categories,

  1. natural repellents example – citronella oil, lemongrass oil, neem oil

2. chemical repellents example – DEET (N,N-Diethyl-m-Toluamide), Permethrin.[163]

- **INSECTICIDE TREATED MOSQUITO NETS AND CURTAINS –** effectively utilised to protect infants and night workers who sleep by day. Also effective for people who have afternoon sleep.[164]

BIOLOGICAL CONTROL

- **FISH –** larvivorous fish (Gambusiaaffinis and Poeciliareticulata) have been extensively used for the control of An. Stephensi and/or Ae. Aegypti in large water bodies and large water containers in many countries in South East Asia.[165]
- **BACTERIA –**2 SPECIES of endotoxin producing bacteria, Bacillus thuringiensis serotype H-14(Bt.H-14) and Bacillus sphaericus(Bs) are effective mosquito control agents. Products include wettable powders and various slow release formulations including briquettes, tablets and pellets.[166]
- **CYCLOPOIDS –** suitable for large containers which cannot be cleaned regularly (wells, concrete tanks and tyres). They can also be used in conjunction with Bt.H-14. Copepods have role in dengue vector control.
- **AUTOCIDAL OVITRAPS –** It is believed that under certain conditions, this technique could be an economical and rapid means of reducing the natural density of adult females as well as a device for monitoring infestations in areas where some reduction in population densities of the vector have lalready taken place.[167]
- **PLANT BASED REPELLENTS –** flavonoid compounds from Poncirus trifoliate compounds have various activities against different stages of aedes aegypti. Larvicidal and ovicidal activities of extracts of eclipta alba have shown potential for controlling aedes aegypti mosquito.[168]

CHEMICAL CONTROL[160]

- **CHEMICAL LARVICIDING –** limited to domestic use containers that cannot be destroyed, eliminated or otherwise managed. There are three insecticides that can be used for treating containers that hold drinking water.

    1. **Temephos 1% sand granules:**[169] applied to containers using a calibrated plastic spoon to administer dosage of 1ppm.this dosage is found effective for 8-12 weeks, especially in porous earthen jars, under normal water use patterns.
    2. **Insect growth regulators:** interfere with the development of immature stages of the mosquito by interference of chitin synthesis during the molting process in larvae or disruption of pupal and adult transformation processes.in general IGRs may provide long term residual effects (3-6months) at relatively low dosages when used in porous earthen jars.
    3. **Bacillus thuringiensis H-14:** it is entirely safe in umans when the larvicideis used in drinking water in normal dosages**.**[166]

- **SPACE SPRAYS:**involves the application of small droplets of insecticide into the air in an attempt to kill adult mosquitoes. Generally there are 2 forms of space spray that have been used for Ae. Aegypti control, namely thermal fogs and cold fogs.both can be dispensed by vehicle mounted or hand operated machines.[160]
- **Thermal fogs**[170]**-** are produced when a suitable formulation condences after being vapourised at a high temperation. The droplet size of thermal fog is less than 15 microns in diameter.

- **Ultra- low volume (ULV), aerosols (cold fogs) and mists :** ULV involves the application of a small quantity of concentrated liquid insecticides. Droplet size is important and the equipment used should be able to produce droplets in 10-15micron range, although the effectiveness changes little when the droplet size is extended to 5-25microns. Aerosols, mists and fogs may be applied by portable machines, vehicle mounted generators or aircraft equipment.
- House to house application using portable equipment: this equipment is meant for restricted outdoor use and for enclosed spaces of not less than 14 m2.
- Vehicle mounted fogging: vehicle mounted aerosol generators can be used in urban and suburban areas with a good road system. One machine can cover 1500-2000 houses/day. Vehicle should not travel faster than 16kph. Best time of administration is early morning(0600-0830 hours) or late afternoon(1700-1930 hours)[160]

PREVENTION OF DENGUE IN TRAVELLERS

Stayingin screened or airconditioned rooms, spraying theserooms with aerosol bomb insecticides to kill adult mosquitoes indoors, using repellents containing DEET (dimethyl metatoluamide) on skin, and wearing protective clothing treated with similar repellent.[7]

Dengue vaccine–

Although no licensed dengue vaccine is yet available,several vaccine candidates are under development. These include

- Live attenuated virus vaccines,

- Live chimeric virusvaccines,
- Inactivated virus vaccines, and
- Live recombinant, dna and subunit vaccines [171]

Live viral vaccines

These have advanced to clinical trials, but have shown problems, such as unequal immunogenicity of the four serotypes and viral interference among the four serotypes in tetravalent formulations. Non-viral vaccines have also been proposed and developed for safety reasons. This includes subunit vaccines that mostly focused on the E protein or its derivatives. NS1 is another subunit vaccine candidate that it is not a virion associated protein and it has no ADE effects [172].

Live attenuated virus vaccines

Live attenuated virus vaccines contain weakened viruses that still can induce adaptive immune responses to both structural and nonstructural proteins. The replication of live attenuated viruses should be sufficiently restricted to avoid pathological effects. A more modern approach is based on site-directed mutagenesis of the viral genome to cause attenuation. A deletion of 30 nucleotides (△30) in the 3′-untranslated region of DENV4 was first demonstrated to attenuate DENV4, named as DEN4 △ 30 [173], and used in Phase I clinical evaluation. But this strategy resulted in attenuation for DENV1 and DENV4, with retained immunogenicity, it was less successful for DENV2 and DENV3. [174]

Monovalent DENV vaccines (DEN1 △30, DEN2/4 △30, DEN3/4△30 and DEN4 △30) have been tested for attenuation and immunogenicity in animal models and humans, and the attenuated tetravalent DENV vaccine admixtures are currently in Phase I clinical studies [175]

Live chimeric virus vaccines

The most advanced product so far, Sanofi Pasteur's ChimeriVax Dengue tetravalent vaccine (CVD1-4) utilized the licensed YFV 17D vaccine as backbone, each expressing the prM and E genes of one of the four DENV serotypes Pre-clinical studies demonstrated that the tetravalent vaccine is genetically and phenotypically stable [176], less neurovirulent than YFV 17D and immunogenic in monkeys. [177]

In Phase I studies, the tetravalent CVD vaccine appeared safe with relatively low viremia [178]. Phase II study showed only 30 percent effectiveness and efficacies against only DENV1, 3 and 4 serotypes. These results indicate that the Sanofi dengue vaccine still carries the risk of ADE and needs more testing, modification and/or clinical trials especially in dengue-endemic countries.[179]

Inactivated virus vaccines

Inactivated virus vaccines have two advantages over live virus vaccines, i.e. no possibility of reverting to virulence (safety) and relative ease of inducing balanced immune responses (for tetravalent vaccines). A purified, inactivated DENV2 vaccine has been shown to be immunogenic and protective in mice and rhesus monkeys as well as formulated with adjuvants for inducing higher levels of neutralizing Abs and protection against viraemia. [180]

Live recombinant, DNA and subunit vaccines

Recent advances in molecular biology have spurred dengue vaccine efforts using live recombinant, DNA and subunit vaccines. The DENV E protein is used as the major immunogen. Certain live viral vectors, such as adenovirus, alphavirus and vaccinia virus are designed for direct administration to the host and have been engineered to express DENV E protein for further evaluation as vaccines.[181]

In addition, recombinant E proteins expressed from yeast and insect cells have been used to test for immunogenicity and protective efficacy in animal models.[182] Truncated E proteins (DEN-8E) produced for all serotypes have been developed with aluminum hydroxide (adjuvant) as tetravalent vaccine formulations. [183]

NS1 is not a structural component of the virion, and therefore, does not contribute to ADE. Anti-DENV NS1 Abs are potentially protective antibodies since they trigger complement-mediated lysis of DENV-infected cells. Several studies indicated that passive immunization with anti-NS1 Abs, DNA vaccine against NS1 proteins,or recombinant vaccinia virus expressing NS1 and active immunization with NS1 proteins could provide protection in mice against DENV challenge.[184]

Although no licensed dengue vaccine is yet available, the ever-increasing knowledge of dengue pathogenesis, is providing more insights into improved vaccine design. Important aspects of dengue vaccine development include common features such as immunogenicity, reactogenicity and protective efficacy but also dengue unique features such as the heterotypic nature of the virus, the risk of ADE and cross-reactivity with host proteins. Furthermore, all of these aspects should ideally be tempered with considerations of cost and stability.[185]

Dengue vaccine candidates in clinical development

VACCINE TYPE Technological approach	**DEVELOPMENT STAGE** Selected clinical trials	**DEVELOPER**
Live attenuated vaccine YF17D/Den chimeric viruses	**Phase 3** NCT01373281 NCT01374516	Sanofi Pasteur
Live attenuated vaccine Attenuated DEN2 PDK-53 virus & DEN/DEN intertypic chimeric viruses	**Phase 2** NCT01511250	Inviragen
Live attenuated vaccine Targeted mutagenesis of DEN viruses & DEN/DEN intertypic chimeric virus	**Phase 2** NCT01696422	Butantan institute National institute of Allergy & Infectious diseases
Recombinant subunit vaccine Truncated E protein	**Phase 1** NCT01477580	Merck
Purified inactivated virus vaccine	**Phase 1** NCT01702857 NCT01666652	GlaxoSmithKline Oswaldo Cruz Foundation Walter Reed Army Institute of Research
DNA Vaccine Expression of prM & E protein	**Phase 1** NCT01502358	Naval Medical Research Center Walter Reed Army Institute of Research

MATERIALS AND METHODS

SOURCE OF DATA

Patients of both sexes irrespective of age groups attending BLDEAs Shri B M Patil Medical College Hospital and Research Centre, Bijapur

INCLUSION CRITERIA

1. Fever
2. Two or more of following manifestations
    - Headache
    - Retro-orbital pain
    - Myalgia
    - Arthralgia
    - Rash
    - Haemorrhagic manifestation
    - Leucopenia

EXCLUSION CRITERIA

1. Focal source of infection. Ex: otitis media, pneumonia, meningitis
2. Chronic illness including anemia
3. Unstable vital signs

METHOD OF COLLECTION OF DATA

Study design: Cross-sectional study from December 2012 to August 2014.

A total of 90 clinically suspected dengue viral fever satisfying inclusion and exclusion criteria were included in the study.

History was taken from the patients and the haematological findings were noted from the patients case sheets.

Ethical clearance was taken for this study from the Ethical Clearance committee of the university.

Blood samples were collected after taking informed and written consent from study participants or from parents/guardian in case of children.

2ml of Blood was collected following venepuncture with all aseptic precautions in plain red vacutainers in adults as well as children. This was sent to laboratory immediately and the serum was separated.

Serum samples were removed from the clot as soon as possible to avoid haemolysis. Rapid kit test (Dengue Day 1 test kit, J mitra) was performed and the serum was frozen at -20˚C for performing ELISA (NS1, IgM, IgG microlisa , J.mitra, 48 tests kit).

SEROASSAYS:

Dengue Kit test:

Dengue Day 1 Test kit(J.mitra) is a rapid solid phase immunochromatographic test for the qualitative detection of Dengue NS1 Antigen and differential detection of IgM and IgG antibodies to Dengue virus in Human serum/plasma. Dengue Day 1 test kit consists of two device. One for the detection of Dengue NS1 antigen and other for the differential detection of Dengue IgM/IgG antibodies in human serum/plasma. 1 kit was used at a time for single sample.

Dengue day1 test foil pouches & test specimen was kept at room temperature before tests were performed. Once the assay started, the complete procedure was done without interruption.

Dengue NS1 antigen device:

Dengue NS1 Antigen device contains two lines; 'C' (Control line) & "T" (Dengue NS1 Antigen test line). Test line is coated with anti-dengue NS1 Ag.

2 drops of sample was added using dengue antigen test sample dropper to the sample well of antigen device. Reaction was allowed to occur for 20minutes. Results were read at 20 minutes. Dengue NS1 antigen if present in the sample will bind to the anti-dengue NS1 gold colloidal conjugate making antigen antibodies complex. This complex migrates along the membrane to the test region and forms the visible pink line at “T” as antibody-antigen-antibody gold colloid forms.

Dengue IgM/IgG device:

Dengue IgM/IgG contains three lines; "C" (Control line), "M"(IgM test line) & "G"(IgG test line). IgM test line is coated with anti-human IgM and IgG test line is coated with anti-human IgG.

10µl of sample was added to the sample well of antibody device,then 2 drops of dengue antibody assay buffer was added to the buffer well. Results were read at 20minutes. IgG and IgM antibodies if present in the sample will react with anti-human IgM or IgG antibodies coated on the membrane respectively. Colloidal gold complexes containing dengue 1-4 antigens is captured by the bound anti-dengue IgM or IgG on respective test bands located in the test window causing a pale to dark red band to form at the IgG or IgM region of the test device window.

The intensity of the test bands in the respective device will vary depending upon the amount of antigen /antibody present in the sample. The appearance of any pink/ red colour in a specific test region was considered as positive for that particular antigen and/or antibody type (IgG or IgM)

INTERPRETATION OF TEST

1. DENGUE NS1 Antigen device:

   Reactive – if pink coloured line is present in test & control region.

   Non reactive – if pink coloured line is present only in control region.

   Invalid – if neither control line nor test line appears on the membrane.

2. DENGUE IgM, IgG Antibody device

   IgM & IgG Reactive – if pink coloured line is present in test (IgM, IgG region) & control region.

   IgM Reactive – if pink coloured line is present in test (IgM region) & control region.

   IgG Reactive – if pink coloured line is present in test(IgG region) & control region.

   Non reactive – if pink coloured line is present only in control region.

   Invalid – if neither control line nor test line appears on the membrane.

NS1 Ag MICROLISA:

1 ELISA KITs (48 test pack each) was used at a time. The frozen serum samples were allowed to thaw in a vertical position in the rack.

NS1 serotype specific IgG ELISA was performed as follows,

Components & test specimen was kept at room temperature before tests were performed. Once the assay started, the complete procedure was done without interruption.

Stripholder was fitted with 48 number of anti dengue NS1 antibody coated strips.the assay control wells were arranged in vertical configuration.

First 50µl of diluent was added in all wells, then 50 µl of negative control, 50µl of positive control, then 50µl of calibrator was added. Then samples of 50 µl in all the other wells. Meanwhile fresh working conjugate was prepared and 100µl of working conjugate was added in all the wells. Thorough mixing of control and samples with the conjugate was done. Cover seal was applied and incubated at 37˚C for 90min. While being incubated working wash solution was prepared with the reagents. After 90 min of incubation, taking out the plate , washing of the wells 6 times with working wash solution was done. Then 150µl of working substrate solution was added in each well & incubated at room temperature for 30min in dark, then stop solution 100µl was added. Absorbance was read at 450nm within 30minutes in ELISA reader.

Calculation of results:

Test validity- ensure the following is within specified acceptance criteria:

1. Ratio of NC O.D/cut off value must be < 0.50
2. Ratio of PC O.D /cut off value must be >1.5
3. Cut off value must be ≥ 0.20

    A. Cut off value = mean O.D of callibrator$_x$ calibration factor

    B. Sample O.D ratio = $\frac{\text{sample O.D}}{\text{Cut off value}}$

    C. Dengue NS1 Ag units = sample O.D ratio × 10

INTERPRETATION OF RESULTS

1. Negative for dengue NS1 antigen if dengue NS1 Ag units is < 9
2. Equivocal for dengue NS1 antigen if dengue NS1 Ag units is 9 - 11
3. Positive for dengue NS1 antigen if dengue NS1 Ag units is >11

Capture IgM and IgG ELISA:

A modified capture IgM and IgG ELISA is performed to measure the IgM and IgG antibodies in patients infected with dengue.

Dengue IgM MICROLISA:

1 ELISA KITs (48 test pack each) was used at a time. The frozen serum samples were allowed to thaw in a vertical position in the rack.

IgM MICROLISA was performed as follows,

Components & test specimen was kept at room temperature before tests were performed. Once the assay started, the complete procedure was done without interruption.

Stripholder was fitted with 48 number of anti dengue NS1 antibody coated strips.the assay control wells were arranged in vertical configuration.

First 100 µl of negative control, 100µl of positive control, then 100µl of calibrator was added in the initial wells. Then diluent of 100 µl in all the other wells, followed by 1µl of sample. Cover seal was applied and incubated at 37˚C for 60min. while being incubated working wash solution and working conjugate was prepared with the reagents. After 60 min of incubation, taking out the plate, washing of the wells 5times with working wash solution was done. Then 100µl of working conjugate solution was added in each well except the first well with negative control, applied cover seal & incubated at 37˚C for 60min, then aspirated and washed 5times

again. 100µl of working substrate solution was then added in all the wells and incubated at room temperature for 30min in dark. then 50µl of stop solution was added . Absorbance was read at 450nm within 30minutes in ELISA reader.

Calculation of results:

Test validity- ensure the following is within specified acceptance criteria

1. Ratio of NC O.D/cut off value must be < 0.3
2. Ratio of PC O.D /cut off value must be >1.1
3. Cut off value must be ≥ 1.5 × NC O.D

    A. Cut off value = mean O.D of callibrator$_x$ calibration factor

    B. Sample O.D ratio = $\frac{\text{sample O.D}}{\text{Cut off value}}$

    C. Dengue IgM units = sample O.D ratio × 10

INTERPRETATION OF RESULTS

1. Negative for dengue IgM antibodies if dengue IgM units is < 9
2. Equivocal for dengue IgM antibodies if dengue IgM units is 9 - 11
3. Positive for dengue IgM antibodies if dengue IgM units is >11

Dengue IgG MICROLISA:

2 ELISA KITs (48 test pack each) were used.1 kit was used at a time. The frozen serum samples were allowed to thaw in a vertical position in the rack.

IgG MICROLISA was performed as follows,

Components & test specimen was kept at room temperature before tests were performed. Once the assay started, the complete procedure was done without interruption.

Stripholder was fitted with 48 number of anti dengue NS1 antibody coated strips. The assay control wells were arranged in vertical configuration.

First 100 µl of negative control, 100µl of positive control, then 100µl of calibrator was added in the initial wells. Then diluent of 100 µl in all the other wells, followed by 1µl of sample. Cover seal was applied and incubated at 37˚C for 60min. while being incubated working wash solution and working conjugate was prepared with the reagents. After 60 min of incubation, taking out the plate , washing of the wells 5times with working wash solution was done. Then 100µl of working conjugate solution was added in each well except the first well with negative control , applied cover seal & incubated at 37˚C for 60min, then aspirated and washed 5times again. 100µl of working substrate solution was then added in all the wells and incubated at room temperature for 30min in dark. then 50µl of stop solution was added . Absorbance was read at 450nm within 30minutes in ELISA reader.

Calculation of results:

Test validity- ensure the following is within specified acceptance criteria

1. Ratio of NC O.D/cut off value must be < 0.3
2. Ratio of PC O.D /cut off value must be >1.1
3. Cut off value must be ≥ 1.5 × NC O.D

A. Cut off value = mean O.D of callibrator$_x$ calibration factor

B. Sample O.D ratio = $\frac{\text{sample O.D}}{\text{Cut off value}}$

C. Dengue IgG units = sample O.D ratio × 10

INTERPRETATION OF RESULTS

1. Negative for dengue IgG Antibodies if dengue IgG units is < 9
2. Equivocal for dengue IgG Antibodies if dengue IgG units is 9 - 11
3. Positive for dengue IgG Antibodies if dengue IgG units is >11

STATISTICAL METHODS APPLIED

1. Following statistical tests were used to compare the results in the present study

   i) Diagrammatic presentation.

   ii) Mean ± S D

   iii) Percentage

- Sensitivity, Specificity, Positive predictive value, Negative predictive value was calculated.
- Statistical analysis was done by software-SPSS17 Version.

RESULTS

Table 1: Agewise distribution of cases w.r.t sex

Age	Total cases	Male	Female
0-10yrs	16 (17.7%)	13 (22.03%)	3 (9.6%)
11-20yrs	21 (23.3%)	12 (20.33%)	9 (29.03%)
21-30yrs	21(23.3%)	15 (25.42%)	6 (19.3%)
31-40yrs	09 (10%)	06 (10.1%)	3 (9.6%)
41-50yrs	10 (11.1%)	07 (11.8%)	3 (9.6%)
>50yrs	13 (14.4%)	06 (10.1%)	7 (22.5%)
Total	90	59	31

From Table 1, it shows that majority of the cases i.e. 21 (23.3%) of the dengue suspected cases belonged to age group of 11-20yrs & 21-30yrs followed by 16 (17.7%) cases belonged to age group of 1-10yrs. Among males 15 (25.42%) cases belonged to 21-30yrs of age followed by 13 (22.03%) cases were of age group of 1-10yrs. Among females 9 (29.03%) cases belonged to age group of 11-20yrs followed by 7 (22.5%) belonged to age group of >50yrs.

Table 2: Agewise distribution of dengue cases w.r.t males

Age	Male (n=59)	Dengue positive (n=23)	Dengue negative (n=36)
0-10yrs	13 (22.03%)	7 (30.43%)	6 (16.7%)
11-20yrs	12 (20.33%)	6 (26.08%)	6 (16.7%)
21-30yrs	15 (25.42%)	4 (17.39%)	11 (30.5%)
31-40yrs	06 (10.1%)	2 (8.7%)	4 (11.11%)
41-50yrs	07 (11.8%)	2 (8.7%)	5 (13.88%)
>50yrs	06 (10.1%)	2 (8.7%)	4 (11.11%)

In the Table 2, among the males suspected of dengue infection 7 (30.43%) cases were positive & 6 (16.7%) were negative in the age group of 1-10years followed by 6 (26.08%) cases were positive & 6 (16.7%) were negative in the age group of 11-20years.

Table 3: Agewise distribution of dengue cases w.r.t females

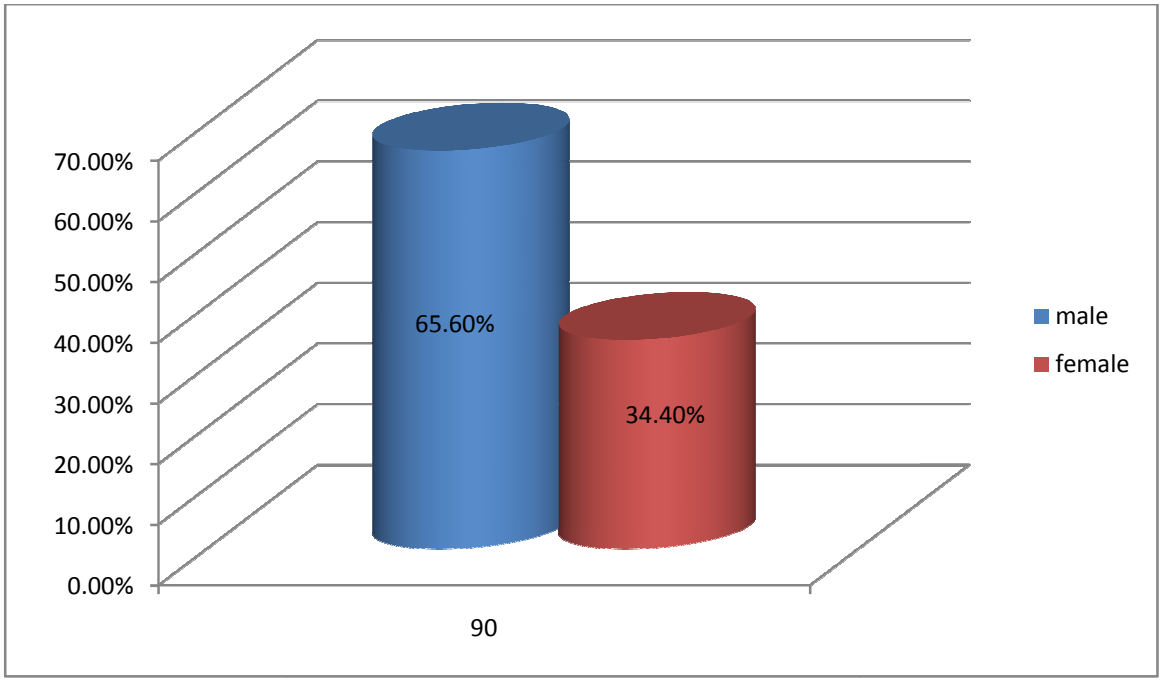
Age	Female (n=31)	Dengue positive (n=16)	Dengue negative (n=15)
0-10yrs	3 (9.7%)	2 (12.5%)	1 (6.67%)
11-20yrs	9 (29%)	6 (37.5%)	3 (20%)
21-30yrs	6 (19.4%)	1 (6.25%)	5 (33.33%)
31-40yrs	3 (9.7%)	2 (12.5%)	1 (6.67%)
41-50yrs	3 (9.7%)	2 (12.5%)	1 (6.67%)
>50yrs	7 (23%)	3 (18.75%)	4 (26.67%)

In the Table 3 , among the females suspected of dengue infection 6(37.5%) cases were positive & 3 (20%) were negative in the age group of 11-20years followed by 3 (18.75%) were positive & 4 (26.67%) were negative in the age group of >50yrs .

Table 4: Sex wise distribution of Dengue cases

Total cases	Male	Female
90	59(65.6%)	31(34.4%)

Graph 1: Sex wise distribution of Dengue cases

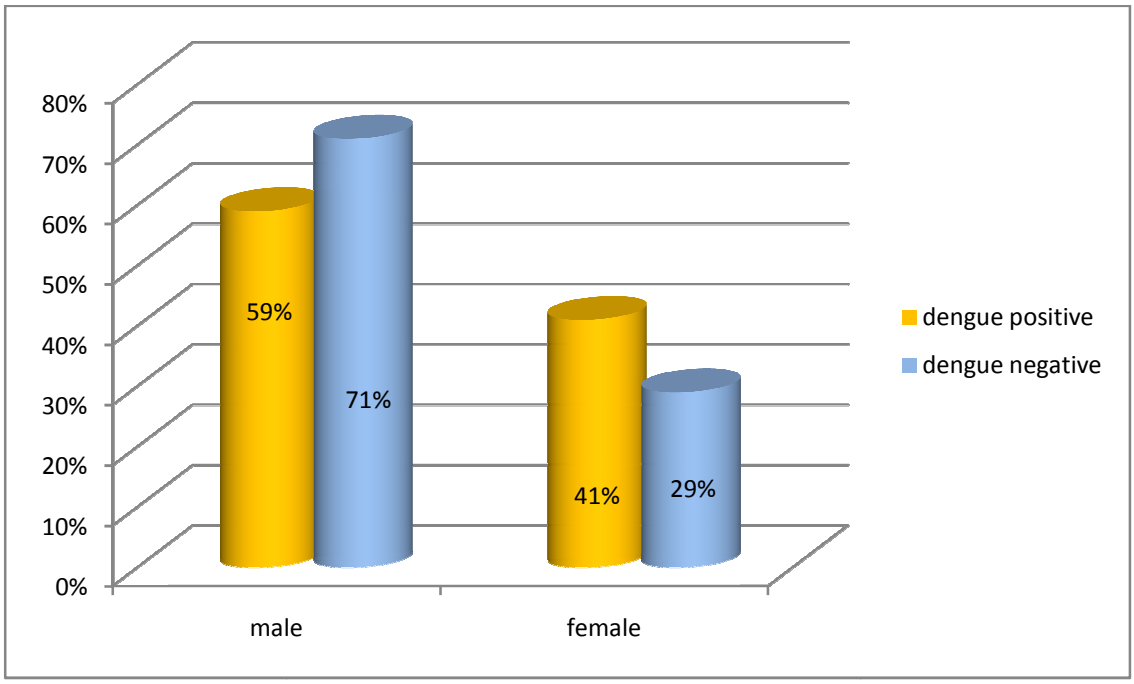


Above table 4 &graph1 shows that out of 90 cases involved in the study, 59 (65.6%) were males & 31 (34.4%) were females.

Table 5: Sexwise distribution of Dengue positive & negative cases

Sex	Dengue positive	Dengue negative	Total
Male	23 (59%)	36 (71%)	59
Female	16 (41%)	15 (29%)	31
Total	39	51	90

Graph 2: sex wise distribution of Dengue positive & negative cases



In the present study out of 59 males, 23 (59%) were positive & 36 (71%) were negative for dengue infection and out of 31 females involved in the study 16 (41%) were positive & 15(29%) were negative for dengue infection as shown in the above table 5 & graph 2.

Table No 6: Analysis of various symptoms

Symptoms	Dengue positive cases (n=39)
Fever	39 (100%)
headache	23 (58.9%)
Joint pain	8(20.5%)
Retro orbital pain	0
Backache	2(5.1%)
haemorrhage	0
Rash	1(2.5%)
Myalgia	28(71.7%)
Hepatomegaly	1(2.5%)
Umbilical tenderness	1(2.5%)
Vomiting	1(2.5%)
Loose stool	1(2.5%)
Abdominal pain	3(7.6%)

Table 6 shows that all the cases had fever (100%). Other common symptoms were myalgia (71.7%) headache (58.9%) & joint pain (20.5%).

Table 7: Association of platelet count with dengue positive cases by Rapid & ELISA test

	Rapid		ELISA		
	Dengue positive	**Percentage**	**Dengue positive**	**Percentage**	**p-value**
<1 lakh/cu mm	21	58.3	23	58.9	0.570
>1lakh/cumm	15	41.7	16	41.1	
Total	36	100	39	100	

Graph 3: Association of platelet count with dengue positive cases by Rapid & ELISA test

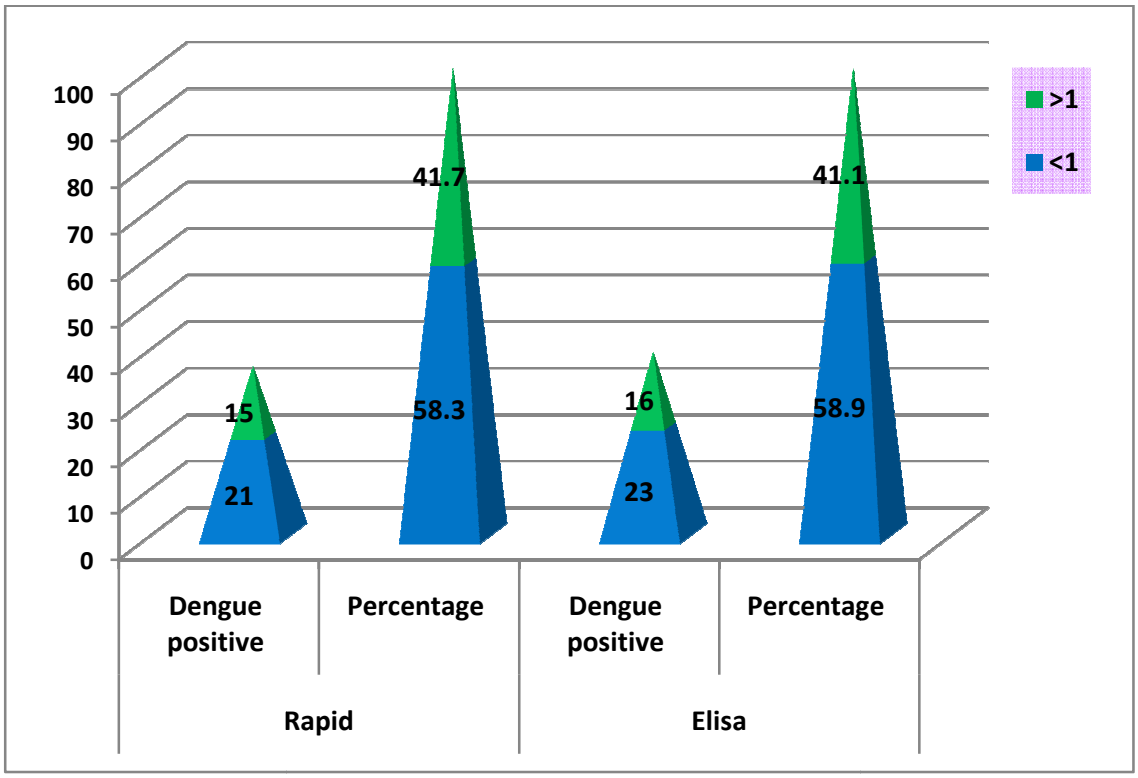
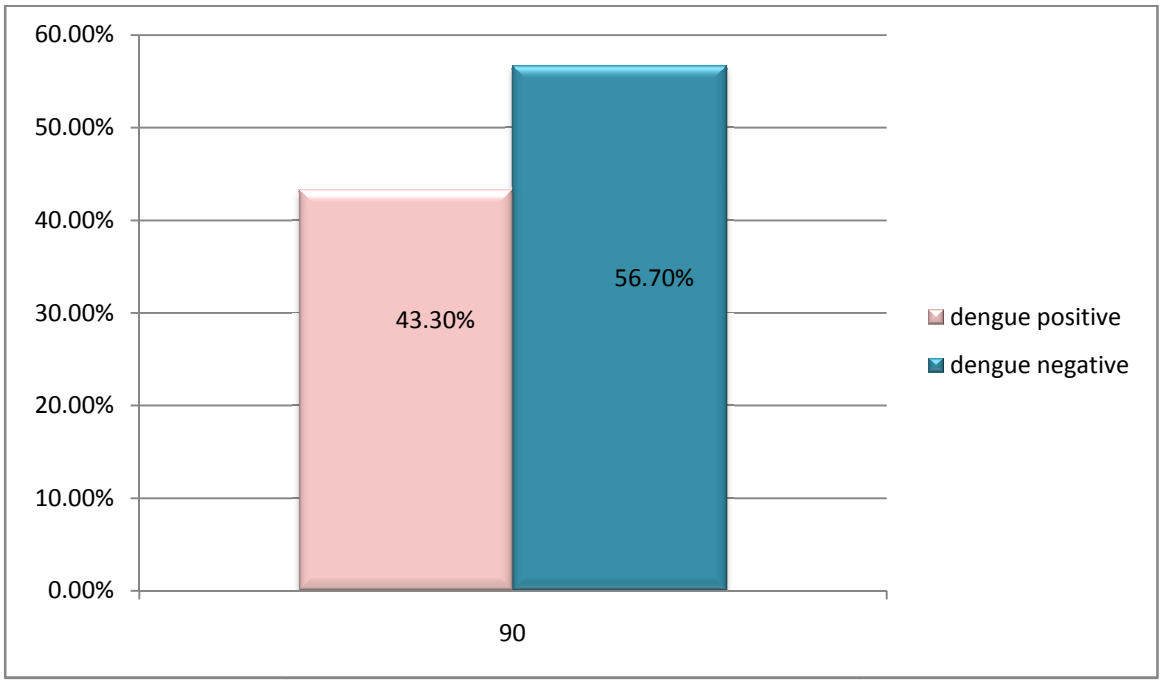


Table 7 & graph 3 shows that thrombocytopenia was seen in 58% of the dengue positive cases tested by rapid & ELISA test, rest of the 42% had normal platelet count, which was not significant.

Table 8:Incidence of Dengue infection

Total cases	Dengue positive	Dengue negative
90	39 (43.3%)	51 (56.7%)

Graph 4: incidence of Dengue infection

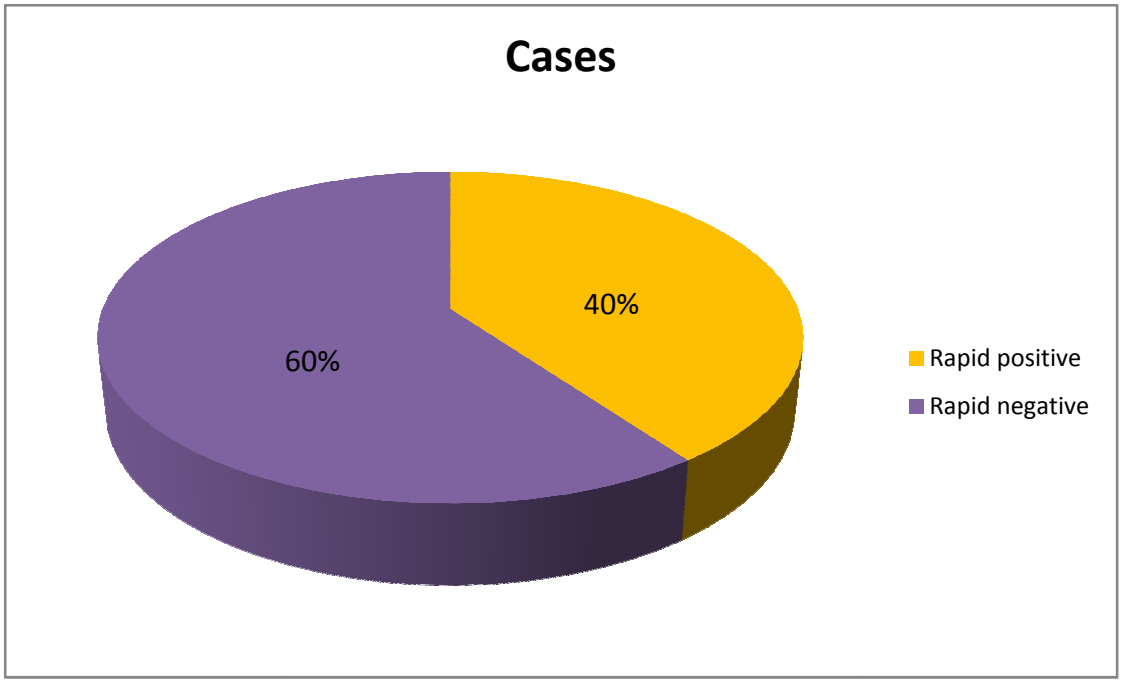


In the present study out of 90 cases involved in the study, 39 (43.3%) cases were positive and 51 (56.7%) cases were negative for dengue infection as shown in table8 & graph4.

Table 9: Distribution of Dengue cases according to Rapid test

Total cases	Rapid test positive	Rapid test negative
90	36 (40%)	54 (60%)

Graph 5: Distribution of Dengue cases according to Rapid test

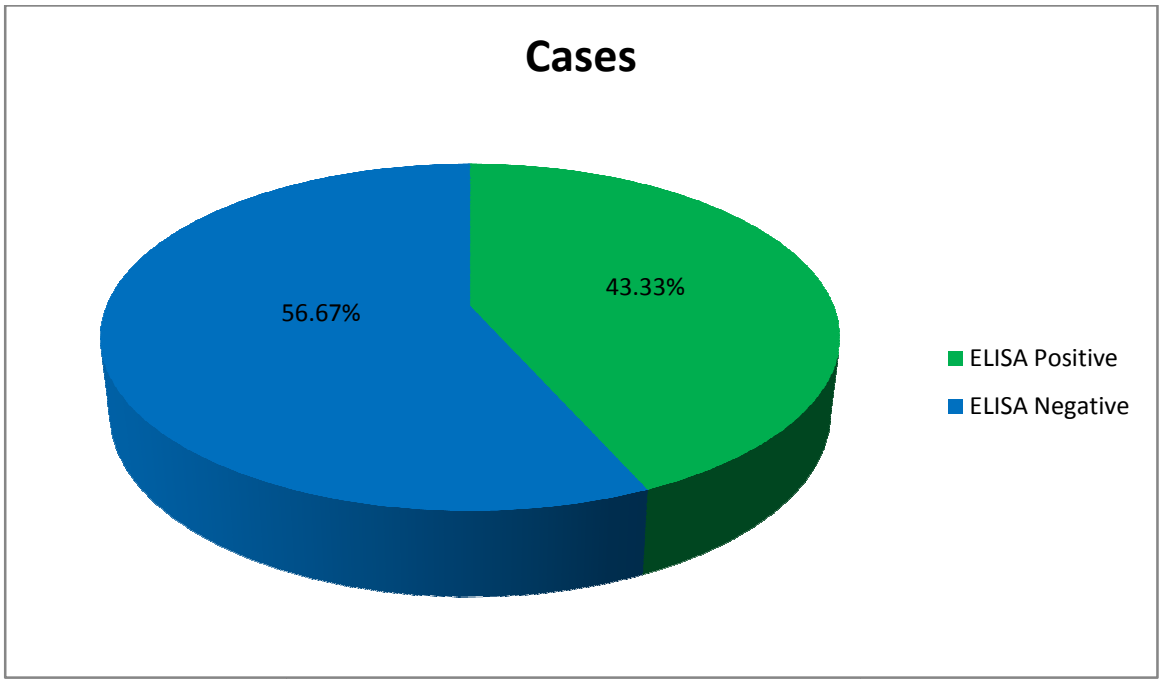


In our study among 90 cases, 36 (40%) were positive & 54(60%) were negative by Rapid test.

Table 10: Distribution of Dengue cases according to ELISA

Total cases	ELISA positive	ELISA negative
90	39 (43.33%)	51 (56.67%)

Graph 6: Distribution of Dengue cases according to ELISA

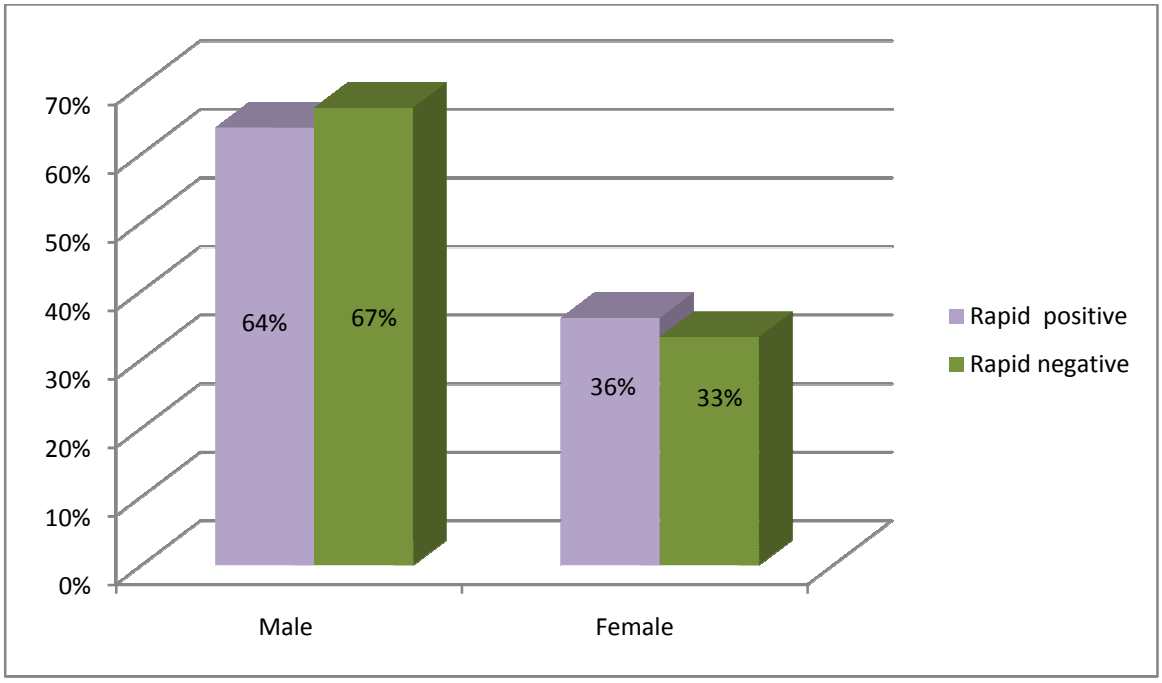


According to table 10 & graph 6, in our study among 90 cases, 39 (43.33%) were positive & 51(56.67%) were negative by ELISA .

Table 11: Sex distribution of dengue cases according to Rapid test

	Total cases	Rapid test	
		Dengue positive	Dengue negative
Male	59	23 (63.8%)	36 (66.7%)
Female	31	13 (36.2%)	18 (33.3%)
Total	90	36	54

Graph 7: Sex distribution of dengue cases according to Rapid test

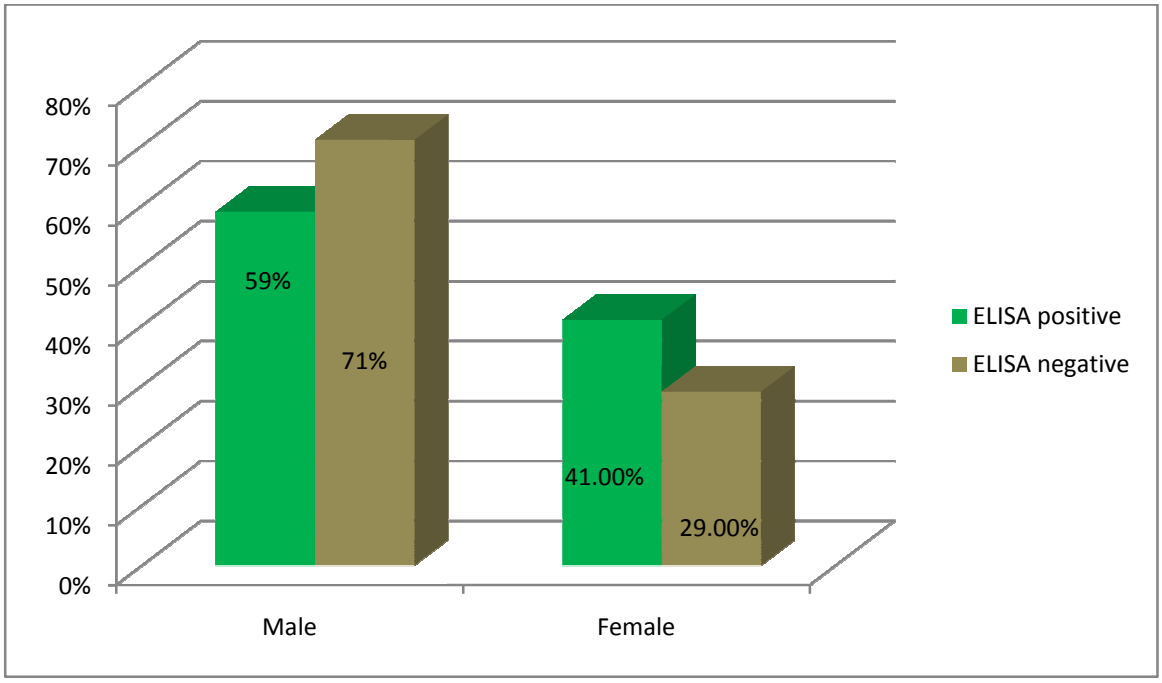


As shown in the above table & graph among the 59 males involved in the study, 23 (63.8%) cases were positive & 36 (66.7%) cases were negative by rapid test and among 31 total females 13 (36.1%) were positive & 18 (33.3%) were negative by rapid test.

Table 12: Sex distribution of dengue cases according to ELISA

		ELISA	
	Total cases	**Dengue positive**	**Dengue negative**
Male	59	23 (59%)	36 (71%)
Female	31	16 (41%)	15 (29%)
Total	90	39	51

Graph 8: Sex distribution of dengue cases according to ELISA test

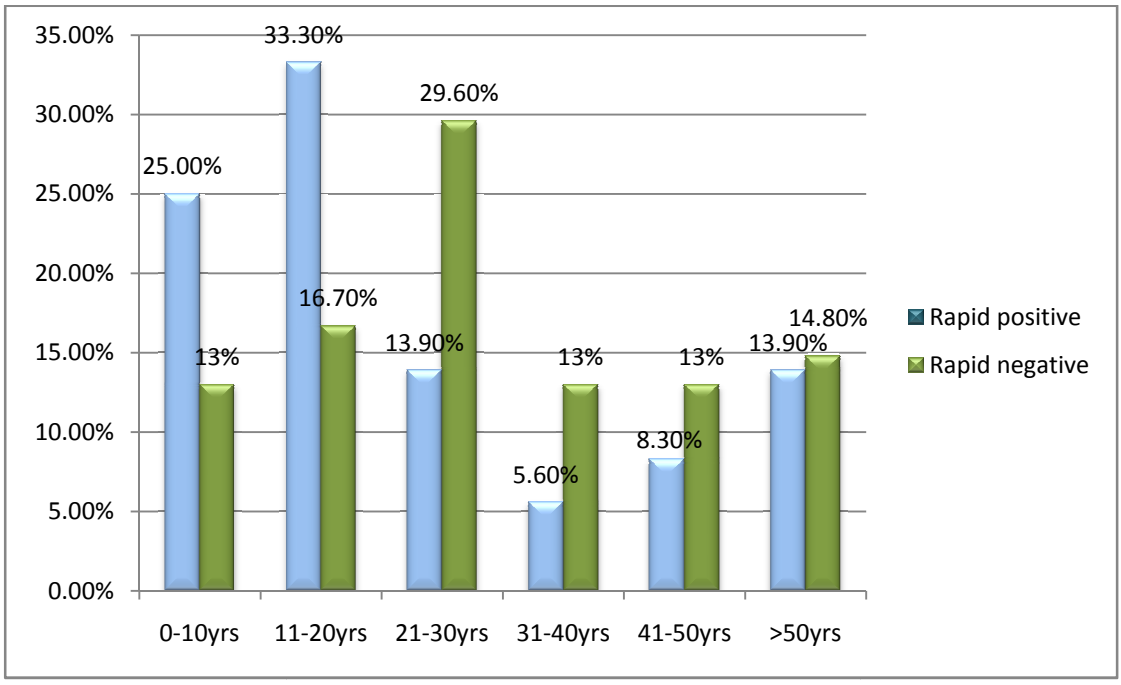


As shown in the above table 12 & graph 8 among the 59 males involved in the study, 23 (59%) cases were positive & 36 (71%) cases were negative by ELISA and among 31 total females 16 (41.%) were positive & 15 (29%) were negative by ELISA.

Table 13: Age wise distribution of dengue cases according to rapid test

Age	Total	Rapid positive	Rapid negative
0-10	16 (17.7%)	9 (25%)	7 (13%)
11-20	21 (23.3%)	12 (33.3%)	9 (16.7%)
21-30	21(23.3%)	5 (13.9%)	16 (29.6%)
31-40	09 (10%)	2 (5.6%)	7 (13%)
41-50	10 (11.1%)	3 (8.3%)	7 (13%)
> 50	13 (14.4%)	5 (13.9%)	8 (14.8%)
	90	36	54

Graph 9: Age wise distribution of dengue cases according to rapid test

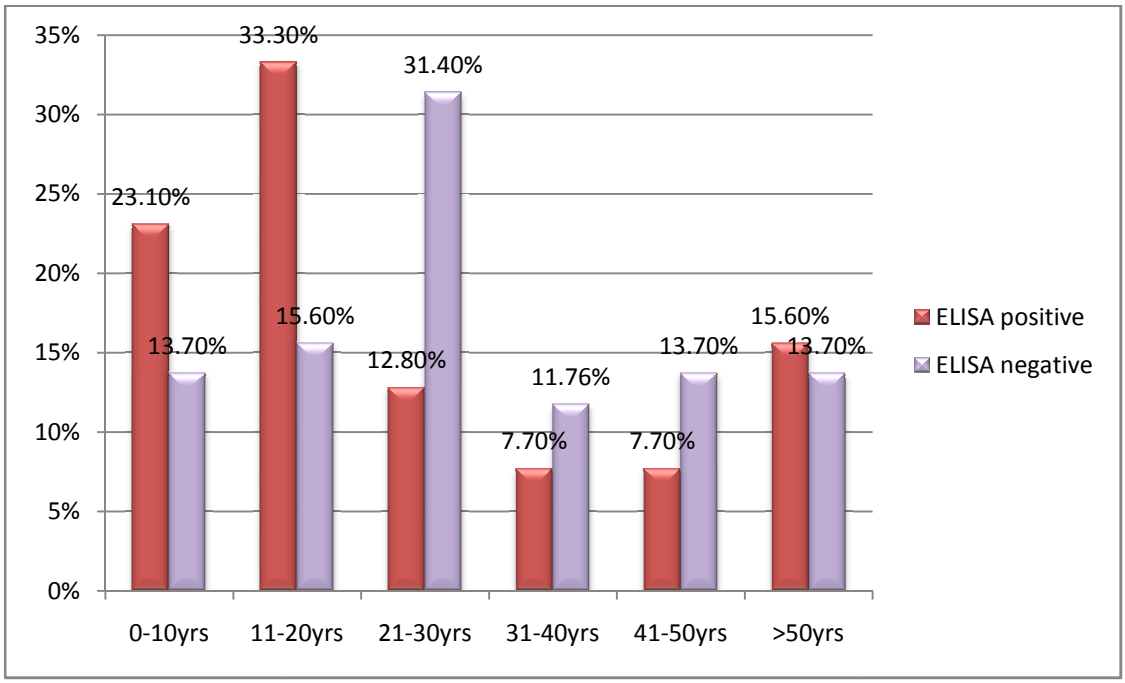


In the present study it showed that among the 21 cases in the age group of 11-20 yrs, 12(33.3%) were positive & 9(16.7%) were negative by rapid test followed by the age group of 0-10yrs which showed 9 (25%) cases were positive & 7 (13%) cases were tested negative by rapid test.

Table 14: Age wise distribution of dengue cases according to ELISA

Age	Total	ELISA positive	ELISA negative
0-10	16 (17.7%)	9 (23.1%)	7(13.7%)
11-20	21 (23.3%)	13 (33.3%)	8(15.6%)
21-30	21(23.3%)	5 (12.8%)	16 (31.4%)
31-40	09 (10%)	3 (7.7%)	6 (11.76%)
41-50	10 (11.1%)	3 (7.7%)	7 (13.7%)
> 50	13 (14.4%)	6 (15.6%)	7 (13.7%)
	90	39	51

Graph 10: Age wise distribution of dengue cases according to ELISA

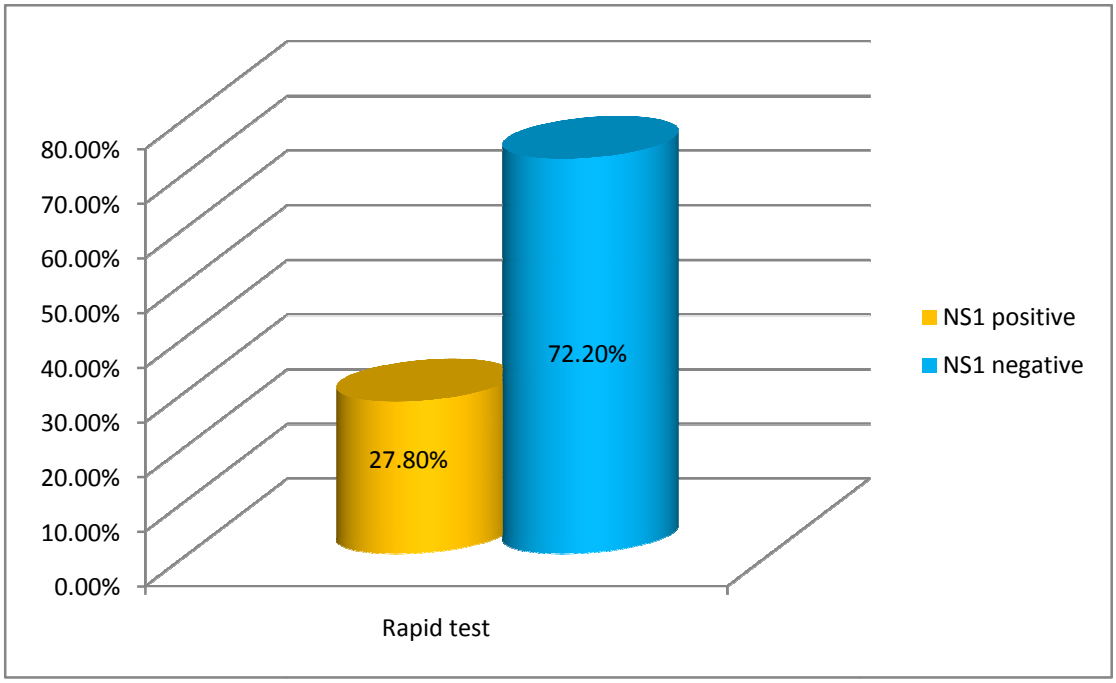


In the present study as shown in table 14 & graph 10, among the 21 cases in the age group of 11-20 yrs, 13(33.3%) were positive & 8(15.6%) were negative by ELISA followed by the age group of 0-10yrs which showed 9 (23.1%) cases were positive & 7 (13.7%) cases were tested negative by ELISA.

Table 15: Distribution of Dengue cases in Rapid test w.r.t NS1 Antigen

NS1 antigen	Rapid test
Positive	25 (27.8%)
Negative	65 (72.2%)
Total	90

Graph 11: Distribution of Dengue cases in Rapid test w.r.t NS1 Antigen

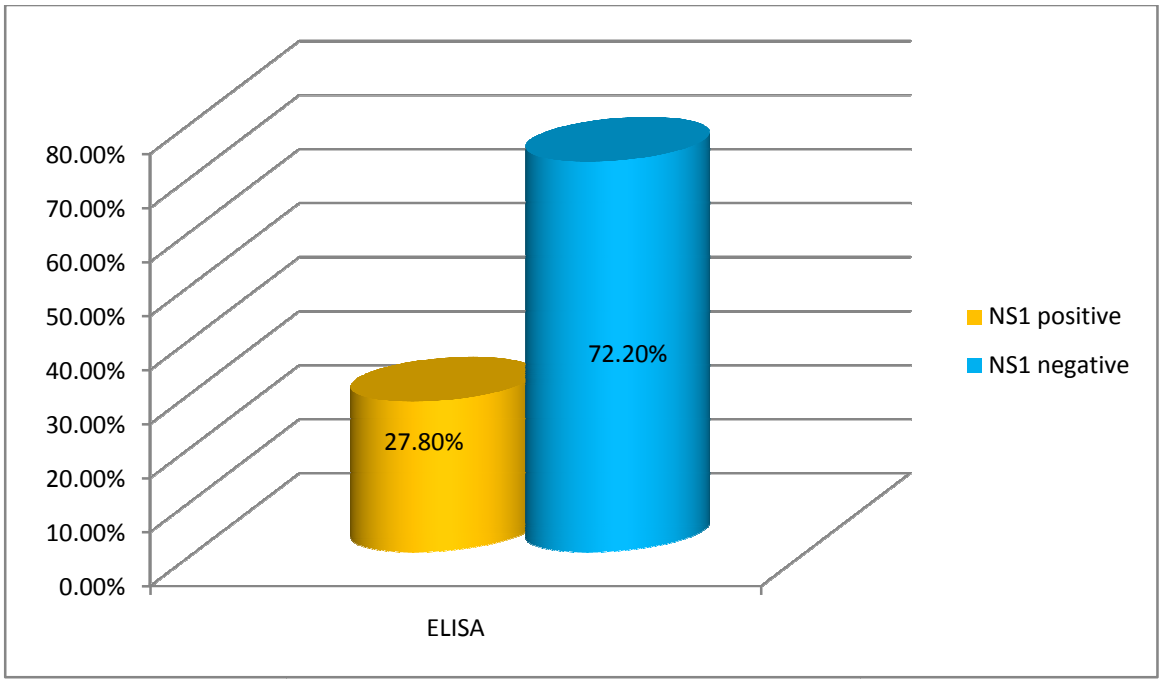


In table 15 & graph 11, the present study out of 90 cases involved, 25(27.8%) were positive and 65 (72.2%) were negative for NS1 antigen by rapid test.

Table 16: Distribution of Dengue cases in ELISA w.r.t NS1 Antigen

NS1 antigen	ELISA
Positive	25 (27.8%)
Negative	65 (72.2%)
Total	90

Graph 12: Distribution of Dengue cases in ELISA w.r.t NS1 Antigen

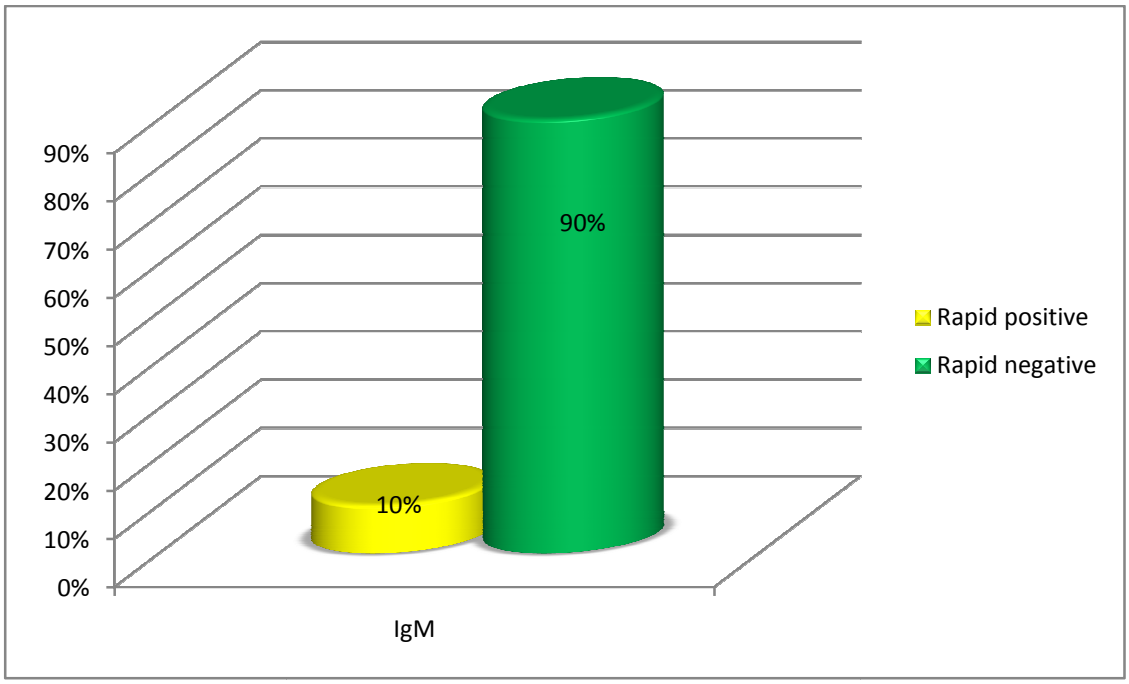


In the present study out of 90 cases involved, 25(27.8%) were positive and 65 (72.2%) were negative for NS1 antigen by ELISA as shown in table16 & graph12.

Table 17: Distribution of dengue cases in Rapid test w.r.t IgM antibodies

IgM Antibody	Rapid test
Positive	9 (10%)
Negative	81 (90%)
Total	90

Graph 13: Distribution of dengue cases in Rapid test w.r.t IgM antibodies

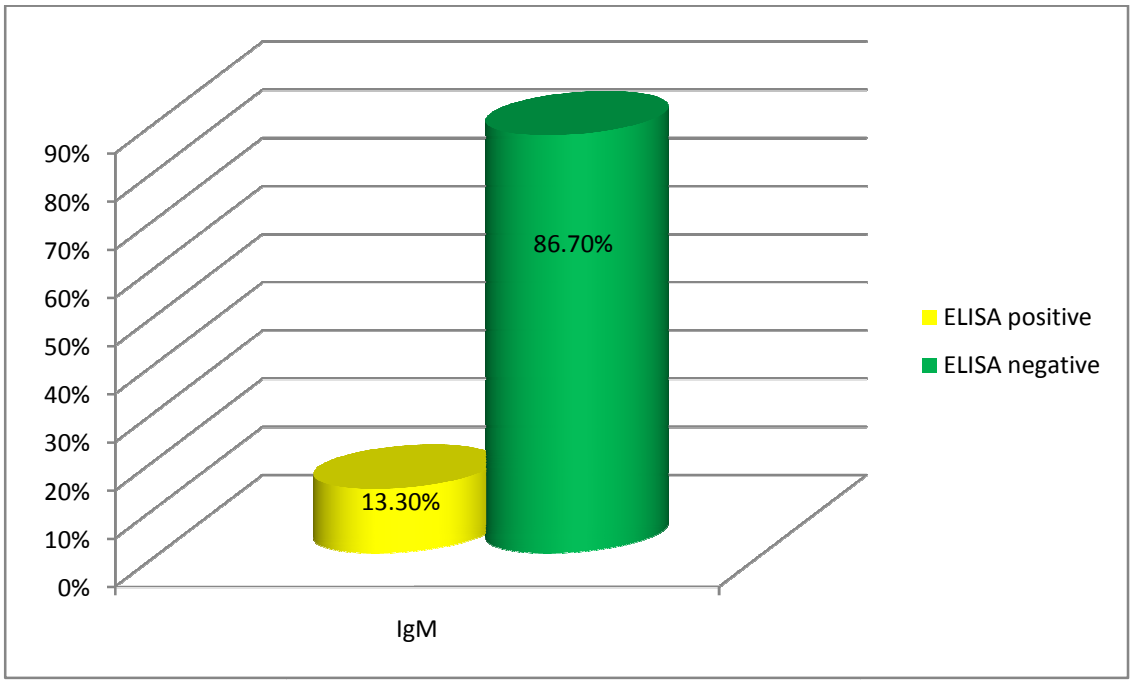


In the present study as shown in table 17 & graph 13 out of 90 cases involved, 9(10%) were positive and 81 (90%) were negative for IgM antibodies by rapid test.

Table 18: Distribution of dengue cases in ELISA w.r.t IgM antibodies

IgM	ELISA
Positive	12 (13.3%)
Negative	78 (86.7%)
Total	90

Graph 14: Distribution of dengue cases in ELISA w.r.t IgM antibodies

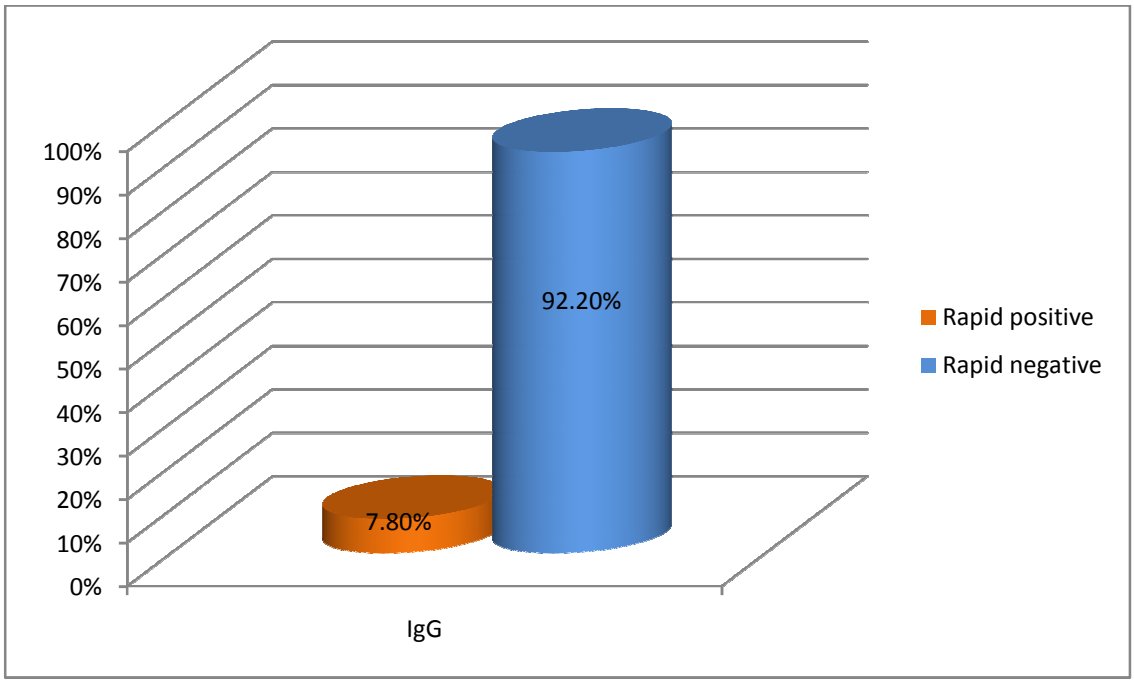


In the present study as shown in table 18 & graph 14 out of 90 cases involved, 12(13.3%) were positive and 78 (86.7%) were negative for IgM antibodies by ELISA.

Table 19: Distribution of dengue cases in Rapid test w.r.t IgG antibodies

IgG Antibody	Rapid test
Positive	7 (7.8%)
Negative	83 (92.2%)
Total	90

Graph 15: Distribution of dengue cases in Rapid test w.r.t IgG antibodies

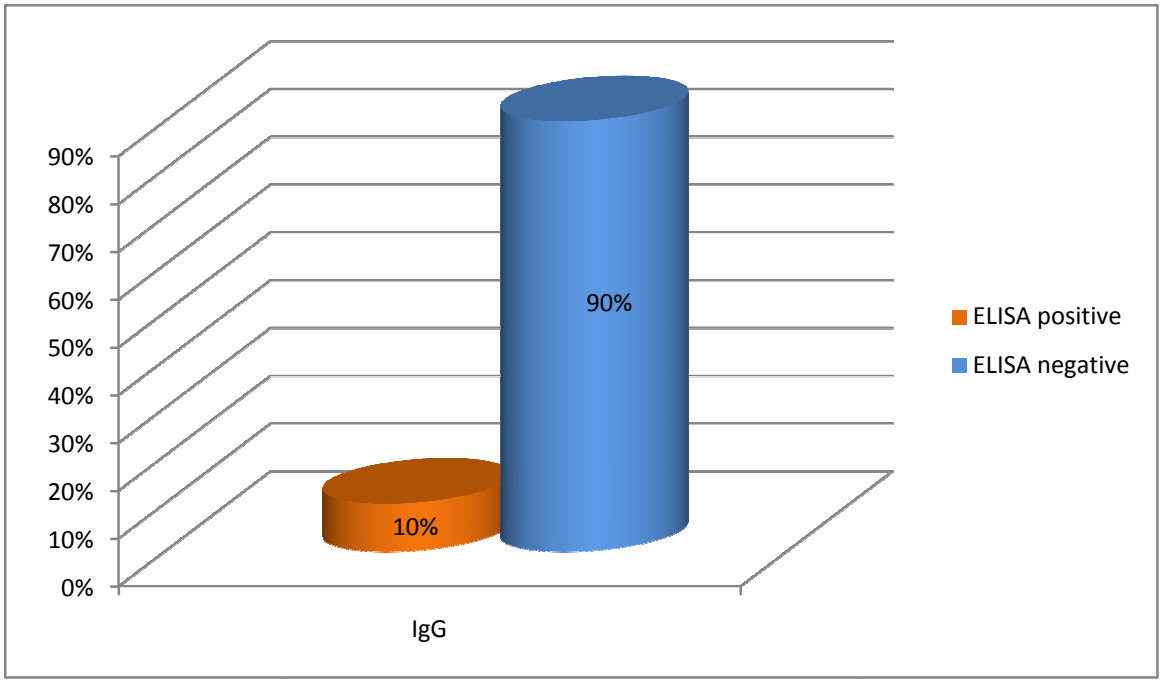


In the present study as shown in table 19 & graph 15 out of 90 cases involved, 7(7.8%) were positive and 83 (92.2%) were negative for IgG antibodies by Rapid test.

Table 20: Distribution of dengue cases in ELISA w.r.t IgG antibodies

IgG Antibody	ELISA
Positive	9 (10%)
Negative	81 (90%)
Total	90

Graph 16: Distribution of dengue cases in ELISA w.r.t IgG antibodies



In the present study as shown in table 20 & graph 16 out of 90 cases involved, 9(10%) were positive and 81 (90%) were negative for IgG antibodies by ELISA.

Table 21: Sensitivity, Specificity. PPV, NPV, Positive Likelihood Ratio, Negative Likelihood Ratio of rapid test

	Value	**95% CI**
Sensitivity	92.31%	79.11% to 98.30%
Specificity	100%	92.95% to 98.30%
Positive predictive value	100%	90.17% to 100%
Negative predictive value	94.44%	84.59% to 98.78%
Positive Likelihood Ratio	0	--------
Negative Likelihood Ratio	0.08	0.03 to 0.23

The present study shows that rapid test has sensitivity of 92% and specificity of 100%, positive predictive value of 100%, Negative predictive value of 94.4% & negative likelihood ratio of 0.08%.

Table 22: Proportions of positive cases in rapid & Elisa test

	Proportion	S.E of Prop	p-value	Result
Rapid	0.40	0.051	0.6818	NS
ELISA	0.43	0.052		

NS- not significant

There is no significant difference between proportions of positive case in rapid test & ELISA, both test gives same result.

DISCUSSION

Dengue virus infection has emerged as the most important and widely spread arboviral disease in the world. Today dengue is endemic in most of the tropical and subtropical countries. The South East Asian regions have recorded increasing incidence of dengue and have contributed to the major portion of global disease burden.

Dengue virus infection was first reported in India from Chennai in 1780. Today dengue virus and all its clinical forms are documented in almost all parts of India.

In the present study, a total of 90 serum samples from patients with clinical features of dengue were analyzed for Dengue rapid test & ELISA for detection of NS1 antigen, IgM & IgG antibodies.

Table 23: Common age groups involved

Sl. No	Author	Year	Place	Age
1	Gore MM[186]	1996	Delhi	5-20 years
2	Baruah J[187]	2002	Manipal	5-20 years
3	Dash PK et al[36]	2003	Gwalior	< 15years
4	Raju BJ et al[188]	2003	Tirupati	0-20 yrs
5	Neerja M et al[189]	2004	Hyderabad	20-39 years
6	Present study	2012-14	Bijapur	11-20 & 20-30yrs

Present study showed that majority of the cases i.e 21 (23.3%) of the dengue suspected cases belonged to age group of 11-20yrs & 21-30yrs. This was

comparable to other studies of Gore MM, Baruah J, Raju BJ et al, Neerja M et al and Dash PK et al.

Table 24: Sex ratio

Sl. No.	Author	Year	Place	M:F
1	Kamal S et al[190]	2002	Warangal	0.72:1
2	Dask PK et al[36]	2003	Gwalior	1.28:1
3	Raju BJ et al[188]	2003	Tirupati	1.5: 1
4	Neerja M et al[189]	2004	Hyderabad	2:1
5	Present study	2012-14	Bijapur	1.4:1

In our study the disease was predominantly seen in case of males (59%) than females (41%) i.e 1.4: 1. This was corresponding to the other studies done by Raju BJ et al, Dash PK et al, Vijayakumar TS et al, Neeraja M et al.

In a study conducted by Kamal S et al females were more commonly affected.

Male preponderance and the age group of 15-30 years indicate more transmission of dengue infections at work sites.[191]

The reason for male preponderance is also due to the greater male exposures to dengue-carrying mosquitoes during daytime hours either at the workplace or while travelling to and from work.[192]

Table 25: Symptoms presented

Sl.no	Study	Fever (%)	Jointpain (%)	Headache(%)	Retroorbital pain (%)	Myalgia (%)	Backache (%)	Rash (%)	Haemorrhage (%)	Hepatomegaly (%)
1	Aggarwal A et al [193] 1996	93	-	-	-	-	-	-	-	72
2	Dash PK et al[36] 2003	100	55	86	-	70	50	56	-	-
3	Neerja M et al[189] 2004	100	15	74	7	53	-	41	7	80
4	Khan E et al[194] 2006	98.3	-	7.5	2.3	23.8	-	37.8	-	-
5	Present study 2012-14	100	20.5	58.9	0	71.7	5.1	2.5	-	2.5

In our study all the cases had fever (100%). Other common symptoms included myalgia (71.7%) headache (58.9%) & joint pain (20.5%). This was comparable with Dash P K et al and Neerja M et al. But in the study conducted by Aggarwal A et al, 93% had fever and 72% had hepatomegaly.

Khan E et al also concluded in their study that 98.3% had fever, followed by rashes & myalgia.

Table 26: Thrombocytopenia in dengue positive cases

Sl. No.	Author	Year	Place	Platelet count <1 lakh/mm3
1	Cherian T et al[195]	1990	Hyderabad	94.7%
2	Singh NP et al[196]	2003	Delhi	61.39%
3	Khan E et al[194]	2006	Thailand	81.4%
4	Present study	2012-14	Bijapur	58%

In our study 58% of dengue positive patients had thrombocytopenia which is comparable to Singh NP et al.

Studies by other authors (Cherian T et al & Khan E et al) showed high association between dengue illness and thrombocytopenia.

Table 27: Dengue incidence

Sl. No	Author	Year	Place	Incidence
1	Huber K et al[197]	1996-97	South Vietnam	62.2%-70%
2	Baruah J[187]	2002-03	Manipal	44%
3	Banerjee G et al[198]	2005	Lucknow	8.69%
4	Present study	2012-14	Bijapur	43.3%

In our study the incidence of Dengue was 43.3%. This was comparable to the study conducted by Baruah J. Low incidence (8.69%) was recorded by Banerjee et al from Lucknow in 2005 & high incidence (62.2%-70%) was recorded by Huber K et al from South Vietnam in 1996-97.

This increase in incidence might be explained by the possible impact of ecological characteristics of the areas on the natural cycles of the arthropod-borne viruses under consideration.[199]

Table 28: Age wise distribution of dengue positive cases by rapid test

Sl. No.	Jalily QA et al[200]	Munir MA et al[201]	Present study
YEAR	2013	2014	2012-14
1-10 yrs	11(10.16%)	17(2%)	9 (25%)
11-20yrs	29(25.4%)	135(16%)	12 (33.3%)
21-30 yrs	48(42%)	294(35%)	5 (13.9%)
31-40yrs	19(17%)	168(20%)	2 (5.6%)
41-50yrs	6(5%)	109(13%)	3 (8.3%)
>50yrs	0	118(14%)	5 (13.9%)

In the present study it showed that in the age group of 11-20 yrs, 12(33.3%) were positive followed by the age group of 0-10yrs which showed 9 (25%) cases were positive by rapid test. But Jalily QA et al found 48(42%) cases positive among 21-30yrs followed by 29(25.4%) cases were positive among 11-20yrs by rapid test.

Study conducted by Munir MA et al showed 294(35%) positive cases among 21-30yrs and 168(20%) positive cases among 31-40yrs by rapid test.

Table 29: Age wise distribution of dengue positive cases by ELISA

Sl. No.	Sarkar A et al[202]	Nepal HP et al[203]	Present study
YEAR	2010	2014	2012-14
1-10 yrs	129(28.92%)	0	9 (23.1%)
11-20yrs	115(25.8%)	7(14%)	13 (33.3%)
21-30 yrs	89(19.95%)	22(44%)	5 (12.8%)
31-40yrs	49(10.98%)	11(22%)	3 (7.7%)
41-50yrs	42(9.4%)	3(6%)	3 (7.7%)
>50yrs	22(5%)	7(14%)	6 (15.6%)

In the present study in the age group of 11-20 yrs, 13(33.3%) were positive by ELISA followed by the age group of 0-10yrs which showed 9 (23.1%) cases were positive.

According to Sarkar A et al ELISA test showed that 129(28.92%) cases were positive among 1-10yrs followed by 115(25.8%) positive cases among 11-20yrs.

Study conducted by Nepal HP et al showed 22(44%) positive cases among 21-30yrs followed by 11(22%) positive cases among 31-40yrs by ELISA.

Table 30: Sexwise dengue positive cases

Sl. No.	Author	Year	Cases	Males dengue positive	Females dengue positive
1	Vijaykumar TS et al[204]	2005	423	135 (32%)	178 (42%)
2	Sood S[205]	2013	2169	292 (70.87%)	120 (29.12)
3	Raju BJ et al[188]	2013	200	49(40.5%)	26 (32.9%)
4	Present study	2012-14	90	23 (58.9%)	16 (41.2%)

90 cases involved in the study, dengue positive cases were 23 (58.9%) males & 16 (41.2%) females which is comparable with study of Vijaykumar TS et al w.r.t females (42%) but males positivity was less i.e 135(32%).

Table 31:Dengue positive cases by Rapid test

Sl. No.	Author	Year	cases	Rapid test Dengue positive	Rapid test Dengue negative
1	Hang VT et al[128]	2009	138	91(66%)	47(34%)
2	Sugimoto M et al[206]	2011	144	12(8.3%)	132(91.6%)
3	Naz A et al[207]	2014	184	103(56%)	81(44%)
4	Present study	2012-14	90	36(40%)	54(60%)

In our study among 90 cases, 36 (40%) were positive & 54(60%) were negative by Rapid test, but according to Hang VT 91 (66%) were positive and 47(34%) were negative, Naz A et al study showed that 103 (56%) were positive and 81(44%)were negative. But Sugimoto M et al study showed low positivity of 8.3% as compared to other studies.

Table 32:Dengue positive cases by ELISA

Sl. No.	Author	Year	cases	ELISA Dengue positive	ELISA Dengue negative
1	Nava M et al[208]	2000	94	44(47%)	50 (53%)
2	Wang SM et al[12]	2010	399	229 (57%)	170 (43%)
3	Rao MRKet al[209]	2014	1980	745 (38%)	1235 (62%)
4	Present study	2012-14	90	39 (43.33%)	51 (56.67%)

In our study among 90 cases, 39 (43.33%) were positive by ELISA which is comparable with the study done by Navu M et al whereas Wang SM et al showed positive cases of 57% and 43% were negative and Rao MRK et al study showed 38% of positive cases and 62% were negative with ELISA test .

Caution should be applied in interpreting tests that are positive to dengue virus IgM & IgG only in areas where dengue virus co circulates with other viruses.[210] This might be the probable reason for the increased ELISA positivity in comparison with rapid test.

Table 33: Sexwise distribution of Dengue cases by rapid test

Sl. No.	Author	Year	Total Male cases	Males positive	Males negative	Total Female cases	Females positive	Females negative
1	Sood S[205]	2013	1356	292 (21.53%)	1064 (78.47%)	813	120 (14.76%)	693 (85.24%)
2	Rao MRK et al [209]	2014	1335	545 (41%)	790 (59%)	645	200 (31%)	445 (69%)
3	Present study	2012-14	59	23 (39%)	36 (61%)	31	13 (42%)	18 (58%)

Among the 59 males involved in our study, 23 (39%) cases were positive & 36 (61%) cases were negative by rapid test and among 31 total females 13 (42%) were positive & 18 (58%) were negative by rapid test which was comparable with thje study conducted by Rao MRK et al. according to Smita Sood only 292(21.53%) of males & 120(14.76%) females were positive .

Table 34: Sexwise distribution of Dengue cases by ELISA

Sl. No	Author	Year	Total Male cases	Males positive	Males negative	Total Female cases	Females positive	Females negative
1	Shah Y et al [211]	2012	148	16(11%)	132 (89%)	141	10 (7%)	131 (93%)
2	Gupta BP et al[212]	2013	163	53(33%)	110 (67%)	112	27(24%)	85(76%)
3	Lavanya R[213]	2014	65	3 (4.6%)	62 (95.4%)	79	1(1%)	78(99%)
4	Present study	2012-14	59	23 (39%)	36 (61%)	31	16 (51.6%)	15 (48.4%)

Among the 59 males involved in the study, 23 (39%) cases were positive & 36 (61%) cases were negative by ELISA which is comparable with Gupta BP et al and among 31 total females in our study 16 (51.6%) were positive & 15 (48.4%) were negative by ELISA. All other studies have shown a low positivity among females as shown in the table above.

Table 35: Distribution of dengue cases by rapid test w.r.t NS1 antigen

Sl. No.	Author	Year	Number of cases	Rapid test NS1 positive	Rapid test NS1 negative
1	Dussart P et al[4]	2008	272	207(76%)	65(24%)
2	Hang VT et al[128]	2009	138	91(66%)	47(34%)
3	Stephen Set al[126]	2014	180	96((53%)	84(47%)
4	Present study	2012-14	90	25(27.8%)	65(72.2%)

Table 36: Distribution of dengue cases by ELISA w.r.t NS1 antigen

Sl. No.	Author	Year	Number of cases	ELISA NS1 positive	ELISA NS1 negative
1	Dussart P et al[4]	2008	272	150(55%)	122(45%)
2	Hang VT et al[128]	2009	138	104(75%)	34(25%)
3	Stephen S et al[126]	2014	180	96(53%)	84(47%)
4	Present study	2012-14	90	25(27.8%)	65(72.2%)

In the present study out of 90 cases involved, 25(27.8%) were positive and 65 (72.2%) were negative for NS1 antigen by rapid test, & 25(27.8%) were positive and 65 (72.2%) were negative for NS1 antigen by ELISA which is similar to Stephen S et al and Hang VT et al but study by Dussart P et al has shown high positivity with rapid test as compared to ELISA.

A factor that may influence the performance of NS1 assays would be the composition of Primary and Secondary dengue infections and health seeking behavior. Hence studies from various populations are needed for a comprehensive understanding of the performance of NS1 assays. [129]

Table 37: Distribution of dengue cases by rapid test w.r.t IgM antibodies

Sl. No.	Author	Year	Number of cases	Rapid test IgM positive	Rapid test IgM negative
1	Palmer CJ et al[136]	1998	80	80(100%)	0
2	Cuzzubo et al[149]	2001	82	61(74.4%)	21(25.6%)
3	Satish N et al[134]	2002	154	35(23%)	119(77%)
4	Present study	2012-14	90	9(10%)	81(90%)

Table 38: Distribution of dengue cases by ELISA w.r.t IgM antibodies

Sl. No.	Author	Year	Number of cases	ELISA IgM positive	ELISA IgM negative
1	Palmer CJ et al[136]	1998	80	78(97.5%)	2(2.5%)
2	Cuzzubo et al[149]	2001	82	61(74.4%)	21(25.6%)
3	Satish N et al[134]	2002	154	32((21%)	122(79%)
4	Present study	2012-14	90	12(13.3%)	78(86.7%)

In the present study out of 90 cases involved, 9(10%) were positive and 81 (90%) were negative for IgM antibodies by rapid test & 12(13.3%) were positive and 78 (86.7%) were negative for IgM antibodies by ELISA. Other studies showed high positive rates with rapid test.

The different commercial kits available have variable sensitivity and specificity . A further challenge in the diagnosis of dengue is the fact that anti-dengue IgM antibodies also cross-react to some extent with other flaviviruses, such as Japanese encephalitis, St Louis encephalitis and yellow fever.[117]

Table 39: Distribution of dengue cases by rapid test w.r.t IgG antibodies

Sl. No.	Author	Year	Number of cases	Rapid test IgG positive	Rapid test IgG negative
1	Cuzzubo et al[149]	2001	82	34(41.4%)	48(58.6%)
2	Chakraverti TK et al[214]	2012	249	101 (40.56%)	148(59.4%)
3	Present study	2012-14	90	7(7.8%)	83(92.2%)

Table 40: Distribution of dengue cases by ELISA w.r.t IgG antibodies

Sl. No.	Author	Year	Number of cases	ELISA IgG positive	ELISA IgG negative
1	Cuzzubo et al[149]	2001	82	27(33%)	55(67%)
2	Chakraverti TK et al[214]	2012	249	105(42.16%)	144(57.8%)
3	Present study	2012-14	90	9(10%)	81(90%)

In the present study out of 90 cases involved, 7(7.8%) were positive and 83 (92.2%) were negative for IgG antibodies by Rapid test & 9(10%) were positive and 81 (90%) were negative for IgG antibodies by ELISA which is similar to Chakraverti TK et al. But Cuzzubo et al showed high positive rate with rapid test.

Table 41: Sensitivity, Specificity. PPV, NPV of rapid test

	Satish N et al (2003)[134]	Hang VT et al (2009)[128]	Andries AC et al (2012)[215]	Pan-ngum W et al (2013)[216]	Present study (2012-14)
Sensitivity	73%	72.8%	85.7%	87%	92.31%
Specificity	95%	100%	83.9%	82.8%	100%
Positive predictive value (PPV)	-	100%	95.6%	62%	100%
Negative predictive value (NPV)	-	27.6%	59.1%	95.2%	94.44%

The present study shows that rapid test has sensitivity of 92% and specificity of 100%, PPV 100%, NPV 94.4% which is comparable with Hang VT (specificity & PPV). The variations in sensitivity & specificity are comparable with previously published data & this might be caused by different principles of assays, different antigens & conjugates.[148]

Thus in the present study, there is no significant difference between positive cases in rapid test & ELISA, both tests give the same result . Thus in comparison with ELISA based assay, rapid immunochromatographic test assay has more favourable design characteristics such as ease of use, possibility of testing individual samples, rapidity and minimal requirement of laboratory equipments & early diagnosis.[129]

The use of IgM & IgG test parameters with NS1 antigen detection is rational as it would likely provide improved presumptive diagnostic coverage towards the end of acute illness when NS1 levels are declining but dengue virus specific IgM & IgG titres are climbing.[217]

Thus rapid test is an effective tool if when used in combination with ELISA in suspected cases, thus improving the diagnostic algorithm contributing significantly to clinical treatment and to control dengue viral infections.

SUMMARY AND CONCLUSION

SUMMARY :

- According to WHO criteria 90 cases were selected for the study.
- Incidence of dengue virus infection was 43.3% .
- Most common age group affected was 11-20 years & 21-30yrs.
- Disease was more common in males than in females (1.4:1).
- All the cases had complaints of fever (100%). Other commonly encountered symptoms were myalgia (71.7%) headache (58.9%) & joint pain (20.5%).
- No single sign or symptom was found to be statistically significant. Combinations of signs & symptoms should be considered for correct clinical diagnosis of dengue.
- Thrombocytopenia was seen in 58% of dengue positive patients.
- 12(33.3%)cases were positive by rapid test & 13(33.3%) were positive by ELISA in the age group of 11-20 yrs followed by 9 (25%) cases positive by rapid test and 9(23.1%) were positive by ELISA in the age group of 0-10yrs .
- Among dengue positive 39 cases , 23(59%) were males and 16 (41%) were females.
- Among 90 cases, 36 (40%) were positive & 54(60%) were negative by Rapid test and 39 (43.33%) were positive & 51 (56.67%) were negative by ELISA.
- Among the 59 males which were studied, 23 (63.8%) cases were positive & 36 (66.6%) were negative by rapid test , 23 (59%) cases were positive & 36 (71%) cases were negative by ELISA.
- Among 31 total females, 13 (36.1%) were positive & 18 (33.3%) were negative by rapid test & 16 (41%) were positive & 15 (29%) were negative by ELISA.

- Out of 90 cases studied, 25(27.8%) were positive and 65 (72.2%) were negative for NS1 antigen by rapid test, & 25(27.8%) were positive and 65 (72.2%) were negative for NS1 antigen by ELISA.
- 9(10%) were positive and 81 (90%) were negative for IgM antibodies by rapid test & 12(13.3%) were positive and 78 (86.7%) were negative for IgM antibodies by ELISA.
- 7(7.8%) were positive and 83 (92.2%) were negative for IgG antibodies by Rapid test & 9(10%) were positive and 81 (90%) were negative for IgG antibodies by ELISA.
- Rapid test has sensitivity of 92%, specificity of 100% , PPV 100% & NPV 94.4%.
- Rapid test has good specificity of 100%, which makes it suitable for screening of dengue infection.

CONCLUSION :

The commercially available rapid immunochromatographic test device can be used as a screening device during dengue outbreaks. It should not be used as a standalone device for diagnosis of dengue. It is recommended that highly suspicious cases should be subjected to tests with higher degree of accuracy (ELISA).

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PROFORMA

NAME : OP/IP NO :

AGE : UNIT/DEPARTMENT:

SEX : DATE :

ADDRESS : LAB NO:

Chief complaints:

1. Fever :

2. Headache:

3. Joint Pain:

4. Retro-obital Pain:

5. Backache:

6. Hemorrhagic manifestation:

7. Rash:

8. Myalgia:

9. Others:

Present history:

Past history:

Treatment history:

Personal history:

EXAMINATION:

Pulse: RR: BP:

Temperature:

1. PA - Splenomegaly:

- Hepatomegaly:

2. CVS:

3. CNS:

4. Respiratory System:

INVESTIGATIONS:

CBC:

DENGUE RAPID TEST: NS1- IgM- IgG-

DENGUE ELISA: NS1- IgM- IgG-

PLATELET COUNT:

CONSENT FORM

TITLE : COMPARISON OF RAPID IMMUNOCHROMATOGRAPHIC METHOD AND ELISA FOR EARLY DIAGNOSIS OF DENGUE VIRUS INFECTION

Dr. Ronni Mol P
P.G. in Microbiology

I have been informed about the research topic. I am ready to take part in the study and give the samples.

Signature

ನನಗೆ ಈ ಸಂಶೋಧನೆಯ ಬಗ್ಗೆ ಸಂಪೂರ್ಣ ಮಾಹಿತಿ ನೀಡಲಾಗಿದೆ. ನಾನು ಸ್ವಇಚ್ಛೆಯಿಂದ ಸ್ಯಾಂಪಲ್ ಕೊಡಲು ಸಿದ್ಧವಾಗಿದ್ದೇನೆ.

ಸಹಿ

मुझे इस रिसर्च के बारे में समझाया गया है। मै अपने मन से स्यांपल देने के लिए तैयार हूं।

सही

ETHICAL CLEARANCE CERTIFICATE

B.L.D.E. UNIVERSITY'S

SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR-586 103

INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 18-10-2012 *at* 3-30pm *to scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected & revised version synopsis of the Thesis has been accorded Ethical Clearance.*

Title "Comparison of rapid Immunochromatographic method and Elisa for detection of NS1, IgM, IgG for early diagnosis of dengue virus infections"

Name of P.G. student Dr Ronni Mol P.
Microbiology

Name of Guide/Co-investigator Dr Aparna Takpere
Prof of Microbiology

DR.TEJASWINI. VALLABHA
CHAIRMAN
INSTITUTIONAL ETHICAL COMMITTEE
BLDEU'S, SHRI.B.M.PATIL
MEDICAL COLLEGE, BIJAPUR.

Following documents were placed before E.C. for Scrutinization
1) Copy of Synopsis/Research project.
2) Copy of informed consent form
3) Any other relevant documents.

KEY TO MASTER CHART

Sl.no	Serial number
Op	Outpatient
In	Inpatient
R.pain	Retroorbital pain
Temp	Temperature
SBP	Systolic Blood Pressure
DBP	Diastolic Blood Pressure
S/S	Signs & Symptoms
LS	Loose stools
V	Vomiting
UT	Umbilical tenderness
A.pain	Abdominal pain
H	Hepatomegaly
P	Present
0	Absent
ELISA	Enzyme linked immunosorbent assay

MASTER CHART

Sl no	Op/Ip	Sex/age	Lab no	Fever	Headache	Joint pain	R.pain	Backache	Haemorrhage	Rash	Myalgia	Platelet count	Temp	Pulse	SBP	DBP	S/S	NS1 rapid test	IgM rapid test	IgG rapid test	NS1 ELISA	IgM ELISA	IgG ELISA
1	339792	32yrs/M	788	p	P	0	0	0	0	0	P	2.5	102	96	106	70	H	0	0	0	0	0	0
2	28736	26/M	793	p	P	0	0	0	0	0	P	3.34	38	102	120	70	0	0	0	0	0	0	0
3	345003	51/F	802	p	P	0	0	0	0	0	P	0.3	38	84	120	80	0	0	0	P	0	0	P
4	30042	45/m	825	p	P	0	0	0	0	0	P	1.44	38.6	88	100	70	0	0	0	0	0	0	0
5	29926	7/m	833	p	P	0	0	0	0	0	P	0.45	38	88	90	60	0	P	0	0	P	P	0
6	30279	21/f	4074	p	P	0	0	0	0	0	0	1.6	38	82	100	50	0	0	0	0	0	0	0
7	30534	34/m	4073	p	P	0	0	P	0	0	P	0.6	37.8	88	100	60	0	P	P	0	P	p	0
8	30613	45/m	4079	p	P	0	0	P	0	0	P	0.75	38	90	120	80	UT	0	P	0	0	p	0
9	30778	22/m	837	p	0	0	0	0	0	0	P	0.85	38.2	88	100	60	0	P	0	0	P	0	P
10	1069	77/f	60	p	0	0	0	0	0	0	0	0.9	38	90	130	80	0	0	0	0	0	P	0
11	24887	17/m	3311	p	P	0	0	0	0	0	0	0.9	38	86	118	80	0	0	P	0	0	P	0
12	24544	28/f	3271	p	0	0	0	0	0	0	0	2.9	37.2	86	114	76	0	0	0	0	0	0	0
13	24114	18/f	3226	p	P	P	0	0	0	0	P	0.9	38.2	80	110	60	0	0	p	0	0	P	0
14	24034	29/m	3221	p	0	0	0	0	0	0	0	1.98	38	86	110	60	0	0	0	0	0	0	0
15	237711	60/m	650	p	0	0	0	0	0	0	P	1	37.8	82	120	70	0	P	0	P	P	0	P
16	23712	32/f	649	p	P	0	0	0	0	0	P	1	37.6	90	110	60	H	0	0	0	0	0	P
17	23659	21/m	3183	p	0	0	0	0	0	0	0	1.02	37.2	86	124	86	0	0	0	0	0	0	0
18	22482	45/m	3064	p	0	0	0	0	0	0	0	1.06	38.2	90	110	70	0	P	0	P	P	0	P
19	275909	53/f	593	p	P	0	0	P	0	0	P	1.1	37.8	90	110	60	0	0	0	0	0	0	0
20	22457	26/f	3063	p	P	0	0	0	0	0	P	1.2	38	90	116	70	0	P	0	0	P	0	0
21	22366	70/f	591	p	0	0	0	0	0	0	0	1.2	37.6	80	130	90	A.pain	0	P	0	0	P	0
22	22038	21/m	583	p	0	0	0	0	0	0	0	0.82	37.8	86	10	80	0	P	0	0	P	0	0
23	26087	4/m	598	p	P	0	0	0	0	1	P	1.2	39.6	120	120	80	V	p	0	0	P	0	0
24	27599	7/m	692	p	0	0	0	0	0	0	P	0.9	38.1	88	90	50	H	P	0	0	P	0	0
25	28452	14/m	787	p	0	0	0	0	0	1	P	1.2	38.1	82	110	70	0	0	0	0	0	0	0

Sl no	Op/Ip	Sex/age	Lab no	Fever	Headache	Joint pain	R.pain	Backache	Haemorrhage	Rash	Myalgia	Platelet count	Temp	Pulse	SBP	DBP	S/S	NS1 rapid test	IgM rapid test	IgG rapid test	NS1 ELISA	IgM ELISA	IgG ELISA
26	28594	13/f	3913	p	0	0	0	0	0	0	0	1.25	39.1	110	90	70	0	0	0	0	0	P	0
27	12925	50/m	1057	p	P	0	0	0	0	0	P	1.3	38	86	130	90	A.pain	0	0	0	0	0	0
28	27782	6/f	778	p	0	P	0	0	0	0	P	1.33	37.6	76	120	70	0	P	0	0	P	0	0
29	15489	6/m	239	p	0	0	0	0	0	0	0	1.36	37.8	72	120	78	0	P	0	0	P	0	0
30	27077	50/m	3586	p	0	0	0	0	0	0	P	1.37	37.6	78	118	72		0	0	0	0	0	0
31	24816	51/m	773	p	0	0	0	0	0	0	0	1.37	37.8	84	130	90	0	0	0	0	0	0	0
32	27766	60/m	769	p	0	0	0	0	0	0	0	1.4	37.4	82	118	64	0	0	0	0	0	0	0
33	27585	37/F	768	p	0	P	0	0	0	0	0	1.4	37.6	82	106	60	0	0	0	0	0	0	0
34	27489	19/F	761	p	0	0	0	0	0	0	P	1.5	36.8	84	110	66	0	0	0	0	0	0	0
35	26418	19/F	734	p	P	P	0	0	0	0	P	0.94	38	84	120	82	0	P	0	0	P	0	0
36	305840	65/F	788	P	P	0	0	0	0	0	P	1.5	37.8	86	120	70	A.pain	0	P	0	0	P	0
37	1242279	26/M	138	P	0	0	0	0	0	0	P	1.5	37.8	70	110	70	0	0	0	0	0	0	0
38	26104	17/M	725	P	0	0	0	0	0	0	P	0.8	38	80	110	80	0	P	0	0	P	0	0
39	17050	28/M	457	P	0	0	0	0	0	0	P	1.5	37.8	78	120	80	0	0	0	0	0	0	0
40	21850	18/M	566	P	0	0	0	0	0	0	P	1.5	38.2	84	110	70	0	P	0	0	P	0	0
41	352024	69/M	817	P	P	P	0	0	0	0	P	1.5	38.2	76	120	80	0	P	0	0	P	0	0
42	19641	18/M	510	P	P	0	0	0	0	0	P	0.56	38	78	120	64	0	P	0	0	P	0	0
43	294794	12/F	669	P	P	0	0	0	0	0	P	0.64	38.6	84	120	72	LS	P	0	0	P	0	0
44	24150	6/M	668	P	P	0	0	0	0	0	P	0.98	36.8	70	110	62	0	P	0	0	P	0	0
45	21832	34/M	573	P	P	0	0	0	0	0	0	1.6	37	74	110	76	0	0	0	0	0	0	0
46	10350	26/F	830	P	P	0	0	0	0	0	0	1.6	38.4	82	110	80	0	0	0	0	0	0	0
47	23330	9/F	638	P	0	0	0	0	0	0	0	1.6	38	82	120	76	0	0	0	0	0	0	0
48	30352	70/F	831	P	P	P	0	0	0	0	P	1.7	38.8	90	120	80	0	0	0	0	0	0	0
49	269030	8/M	574	P	0	0	0	0	0	0	P	1.7	38	86	110	70	0	0	0	P	0	0	P
50	10220	58/M	162	P	0	0	0	0	0	0	0	1.7	37.6	80	112	80	0	0	0	0	0	0	0
51	9824	26/F	801	P	0	0	0	0	0	0	0	1.8	38	80	110	70	0	0	0	0	0	0	0

Sl no	Op/Ip	Sex/age	Lab no	Fever	Headache	Joint pain	R.pain	Backache	Haemorrhage	Rash	Myalgia	Platelet count	Temp	Pulse	SBP	DBP	S/S	NS1 rapid test	IgM rapid test	IgG rapid test	NS1 ELISA	IgM ELISA	IgG ELISA
52	104185	34/M	796	P	P	0	0	0	0	0	P	1.8	37.8	76	110	74	0	0	0	0	0	0	0
53	340880	24/M	792	P	P	0	0	0	0	0	P	1.8	37.8	86	120	76	0	0	0	0	0	0	0
54	24965	45/F	698	P	P	0	0	0	0	0	0	1.9	38	80	120	70	0	0	0	P	0	0	P
55	290037	5/F	652	P	0	0	0	0	0	0	0	0.86	38.2	84	110	8	0	P	0	0	P	0	0
56	15709	13/F	426	P	0	0	0	0	0	0	0	0.4	37.8	80	110	64	0	P	P	0	P	P	0
57	25740	28/M	719	P	P	0	0	0	0	0	0	1.99	38.4	70	110	80	0	0	0	0	0	0	0
58	24591	45/M	703	P	0	0	0	0	0	0	P	2.11	38.4	70	120	70	0	0	0	0	0	0	0
59	306553	14/M	690	P	P	0	0	0	0	0	P	2.21	38	70	120	70	0	0	0	0	0	0	0
60	24387	25/M	677	P	P	0	0	0	0	0	0	2.31	37.8	80	110	80	0	0	0	0	0	0	0
61	23772	8/M	673	P	P	0	0	0	0	0	P	2.39	37.4	70	108	70	0	0	0	0	0	0	0
62	23250	40/F	3142	P	P	0	0	0	0	0	P	2.4	38	78	120	76	0	0	0	0	0	0	0
63	22020	28/M	3022	P	0	0	0	0	0	0	0	2.4	38.2	76	120	82	0	0	0	0	0	0	0
64	24450	48/F	680	P	P	0	0	0	0	0	P	2.41	38.2	78	112	84	0	0	0	0	0	0	0
65	27874	18/M	784	P	0	0	0	0	0	0	0	2.47	37.6	82	110	82	0	0	0	0	0	0	0
66	30590	28/M	4076	P	P	P	0	0	0	0	P	2.5	37.6	78	114	76	0	0	0	0	0	0	0
67	22393	15/M	590	P	0	0	0	0	0	0	0	2.5	37.2	70	100	60	0	0	0	0	0	0	0
68	24642	18/M	4075	P	P	0	0	0	0	0	P	1.2	38.2	70	120	80	0	P	0	0	P	0	0
69	23901	55/M	658	P	P	0	0	0	0	0	P	2.64	37.4	78	110	84	0	0	0	0	0	0	0
70	74487	35/M	597	P	0	0	0	0	0	0	0	2.81	36.8	78	110	80	0	0	0	0	0	0	0
71	26056	5/M	859	P	0	0	0	0	0	0	P	3.04	38.8	88	110	88	0	0	0	0	0	0	0
72	29107	20/M	806	P	P	0	0	0	0	0	P	3.09	38.6	70	120	70	0	0	0	0	0	0	0
73	29848	2/M	822	P	0	0	0	0	0	0	0	3.18	38.2	72	90	70	0	0	0	0	0	0	0
74	22966	20/F	616	P	P	P	0	0	0	0	P	0.96	38.2	78	110	70	0	0	P	0	0	P	0
75	22087	1/M	587	P	0	0	0	0	0	0	0	3.27	37.6	68	114	78	0	0	0	0	0	0	0
76	24584	7/M	3290	P	0	0	0	0	0	0	0	3.28	38	70	110	70	0	0	0	0	0	0	0
77	24362	58/F	3252	P	P	0	0	0	0	0	0	3.31	38.4	78	110	70	0	0	0	0	0	0	0

Sl no	Op/Ip	Sex/age	Lab no	Fever	Headache	Joint pain	R.pain	Backache	Haemorrhage	Rash	Myalgia	Platelet count	Temp	Pulse	SBP	DBP	S/S	NS1 rapid test	IgM rapid test	IgG rapid test	NS1 ELISA	IgM ELISA	IgG ELISA
78	3422	30/M	51	P	P	P	0	0	0	0	P	0.7	38.2	78	120	70	0	P	0	0	P	0	0
79	23774	8/M	654	P	0	0	0	0	0	0	0	3.58	38	70	110	80	0	0	0	0	0	0	0
80	14234	42/F	229	P	P	0	0	0	0	0	P	3.76	38.4	84	120	96	0	0	0	0	0	0	0
81	269030	8/M	574	P	0	0	0	0	0	0	0	3.83	38.2	76	20	70	0	0	0	P	0	0	P
82	20653	35/M	544	P	P	P	0	0	0	0	P	1.4	38	78	130	78	0	P	0	P	P	0	P
83	21859	47/M	2980	P	P	0	0	0	0	0	0	3.95	38.2	80	120	70	0	0	0	0	0	0	0
84	263553	18/M	575	P	P	0	0	0	0	0	P	0.4	37.6	72	100	64	0	P	0	0	P	0	0
85	19994	21/M	522	P	0	0	0	0	0	0	0	4.02	37.2	70	110	80	0	0	0	0	0	0	0
86	334899	21/F	776	P	P	0	0	0	0	0	P	4.08	38	82	110	80	0	0	0	0	0	0	0
87	1033	20/F	18	P	P	P	0	0	0	0	P	2.4	39	90	106	70	A.pain	0	P	0	0	P	0
88	1138	22/M	17	P	P	0	0	0	0	0	P	0.98	38.6	86	110	60	0	P	0	0	P	0	0
89	1328	16/M	79	P	P	0	0	0	0	0	0	0.94	38	80	120	82	V	0	0	0	0	0	0
90	1411	18/F	92	P	0	0	0	0	0	0	0	2.98	37.8	84	110	70	0	0	0	0	0	0	0