““CORRELATION OF THROMBOCYTOPENIA RECOVERY WITH SERUM VITAMIN B12 LEVELS IN DENGUE FEVER”

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LIST OF ABBREVIATIONS USED

ADE	:	ANTIBODY DEPENDENT ENHANCEMENT
ALT	:	ALANINE AMINOTRANSFERASE
ARDS	:	ACUTE RESPIRATORY DISTRESS SYNDROME
AST	:	ASPARTATE AMINOTRANSFERASE
CBC	:	COMPLETE BLOOD COUNT
CF	:	COMPLEMENT FIXATION
CNS	:	CENTRAL NERVOUS SYSTEM
CSF	:	CEREBROSPINAL FLUID
CYD-TDV	:	CHIMERIC YELLOW FEVER VIRUS-DENGUE VIRUS TETRAVALENT DENGUE VACCINE
DEN1	:	DENGUE VIRUS TYPE 1
DHF	:	DENGUE HEMORRHAGIC FEVER
DIC	:	DISSEMINATED INTRAVASCULAR COAGULATION
DF	:	DENGUE FEVER
DSS	:	DENGUE SHOCK SYNDROME
EDS	:	EXPANDED DENGUE SYNDROME
EDTA	:	ETHYLENEDIAMINE TETRAACETIC ACID

EHC : ENTEROHEPATIC CIRCULATION

ELISA : ENZYME LINKED IMMUNO SORBANT ASSAY

FA : FOLIC ACID

GI : GASTROINTESTINAL

HCY : HOMOCYSTEIN

HI : HEMAGGLUTINATION INHIBITION

HOLOTC : HOLO TRANSCOBALMIN

HUS : HEMOLYTIC UREMIC SYNDROME

IF : INTRINSIC FACTOR

IFN : INTERFERON

IL2 : INTERLEUKIN 2

LTC : LONG TERM CARE

MAC-ELISA : IGM ANTIBODY CAPTURE ENZYME LINKED IMMUNO SORBANT ASSAY

MCV : MEAN CELLULAR VOLUME

MMA : METHYMALONIC ACID

MODS : MULTIPLE ORGAN DYSFUNCTION SYNDROME

NASBA : NUCLEIC ACID SEQUENCE BASED AMPLIFICATION

NS1 : NON STRUCTURAL PROTEIN 1

NSAIDS	:	NONSTEROIDAL ANTI INFLAMMATORY DRUGS
NT	:	NEUTRALISATION TEST
NVBDCP	:	NATIONAL VECTOR BORNE DISEASE CONTROL PROGRAMME
ORS	:	ORAL REHYDRATION SOLUTION
PCR	:	POLYMERASE CHAIN REACTION
PPI	:	PROTON PUMP INHIBITOR
RBC	:	RED BLOOD CELL
RDA	:	RECOMMENDED DAILY ALLOWANCE
RDP	:	RANDOM DONOR PLATELET
RDT	:	RAPID DIAGNOSTIC TEST
SAM	:	S ADENOSYL METHIONINE
SDP	:	SINGLE DONOR PLATELET
TNF	:	TUMOR NECROSIS FACTOR
TTP	:	THROMBOTIC THROMBOCYTOPENIC PURPURA
WHO	:	WORLD HEALTH ORGANISATION

ABSTRACT

BACKGROUND: Dengue is one of the most rapidly emerging mosquito – borne viral disease which is now endemic to all the continents with a significant mortality in developing nations. Decrease in platelet count, problems of increased capillary permeability , bleeding manifestations and shock are the few factors which are the major contributor for poor disease outcome. Recovery from thrombocytopenia is seen to be variable in nature in different patients and as we know Vitamin B12 has a role in synthesis of blood cells.

AIMS AND OBJECTIVE: This study is conducted to document correlation between vitamin B12 deficiency and severity of thrombocytopenia, platelet recovery and duration of hospital stay in dengue fever patients.

MATERIALS AND METHODS:A prospective observational study was conducted in BLDE (deemed to be university) Shri B.M Patil medical college Hospital and Research Centre, Vijayapura in patients diagnosed with Dengue Fever. Total blood cell count and serum Vitamin B12 levels were checked on day of admission and daily platelet counts were measured. Patients were divided into three groups on the basis of serum Vitamin B12 levels, Group A (B12 <150 pg/ml) , Group B(B12 < 151 – 300 pg/ml) and Group C (B12 >300 pg/ml). The duration of hospital stay until thrombocytopenia recovery/ Discharge/ Death was observed in all the groups along with daily platelet count.

RESULTS: A total 65 patients were selected initially with Dengue Fever , out of which 4 were excluded as per exclusion criteria and total 61 patients were studied. Most common age group

affected was of < 20 years of age with 42.6 % of patients, with a slight male preponderance as 52.5 % of patients were male. Out of total 67.2 % patients were found to be NS1 antigen positive, 24.6% of patients were IgM positive and 8.2% of patients were IgG positive.

18% of patients were in group A with serum Vitamin B12 < 150 pg/ml , 21.3 % of patients in group B with B12 151 – 300 pg/ml and 60.7 % of patients in group C with B12 levels of >300 pg/ml. Platelet recovery was observed to be slowest in group A with mean 6.455 days, 6.385 in group B and 4.703 in group C and p value of 0.011 (statistically significant).

Duration of hospital stay was observed to be longest in group A with mean 7.45 days, with 6.85 in group B and 4.81 in group C with a statistically significant p value of 0.001.

45.5% of patients of group A, 30.8% patients of group B and 19% patients of group C went for severe thrombocytopenia (platelet count <20,000 cumm).

CONCLUSION : Vitamin B12 deficiency is a contributing factor for the development of severe thrombocytopenia in Dengue Fever patients. Its deficiency delays the recovery of thrombocytopenia in Dengue leading to prolonged Hospital stay creating an extra burden on patient as well as health infrastructure.

KEYWORDS : Dengue Fever , Thrombocytopenia , Vitamin B12

INTRODUCTION

INTRODUCTION

Dengue is one of the most significant arboviral diseases of humans worldwide, It ranks as the most important, rapidly emerged mosquito-borne viral disease and it is endemic in all continents. It is majorly distributed in tropical and subtropical regions that are the natural home for its vector, mosquitoes of the genus Aedes.[1]

The WHO (World Health Organisation) proposed a revised classification of clinical infection: Dengue fever; Dengue with warning signs; and Severe dengue.[1]

Dengue fever causes pyrexia (fever) that lasts 2–10 days, as well as headaches, retroorbital discomfort, joint and muscle pain, and rashes on the skin. Cross-reactive antibodies are produced as a result of secondary infection with a different serotype, which increases the risk of antibody-dependent increament of disease, a type of immunopathology. As a result, recurrent or repeated infection is the most significant risk factor for the catastrophic, often fatal disease.[1]

These are marked by problems of capillary permeability, a decrease in platelet count, disordered blood clotting, severe bleeding, and for DSS(Dengue shock syndrome) alongside systemic shock leading to organ failure.[1]

Recovery from thrombocytopenia can be quick in some dengue patients, but it might take several days in others. During acute hematopoietic stress, there may be other variables that contribute to thrombocytopenia and its delayed recovery.

As a result, identifying and addressing contributing factors may reduce the length of stay in patients with dengue fever.

Sandeep Tak et al. concluded that vitamin B12 deficiency may be a contributing factor to the development of severe thrombocytopenia in dengue fever, especially in the Indian population, in a study that looked at the relationship among severe thrombocytopenia in dengue fever and vitamin B12 levels.[2]

There are no large epidemiological study to assess B12 level in Indian population but few studies suggest that Vitamin B12 deficiency is common in Indian populations with prevalence range of 35% to 60%.[3]

Keeping in view of the present perspective and as this study is not done in this part of the country, it will be conducted to ascertain a relationship between recovery of thrombocytopenia and vitamin B12 levels in dengue fever patients.

AIMS AND OBJECTIVES

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To document correlation between vitamin B12 deficiency and severity of thrombocytopenia, platelet recovery and duration of hospital stay in dengue fever patients.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Dengue fever is the world's fastest-growing and most well-known mosquito-borne viral illness, with a 30 fold rise in global occurrence over the previous five decades. Almost half of the world's population lives in dengue-endemic nations. [1]

According to the World Health Organization (WHO), around 50-100 million new dengue infections occur each year in more than 100 endemic countries, with the number of nations reporting the disease steadily increasing.[1]

Vitamin B12 is necessary for erythropoiesis and thrombopoiesis to occur. Significant thrombocytopenia affects around 10% of people with symptomatic B12 (cobalamin insufficiency).

Sandeep Tak, GeethuChachappan, Jagdambe Singh Rathore, Sheshkaran Singh Charan, RamniwasBijarniya, Manoj Lakhotia, studied the relation of severe thrombocytopenia in dengue fever with vitamin B12 level and concluded thatVitamin B12 deficiency may have a role in the development of severe thrombocytopenia in dengue fever patients, especially in Indians.[2]

Sivaprasad et al conducted a study in South India and concluded that the overall prevalence of B12 deficiency was 35% which was significantly higher in the 21-40 (44%) and 41-60 (40%) age groups when compared with the >60 age group (30%). B12 deficiency was higher in vegetarians(54%) compared to those consuming mixed diet(31%).[3]

Kara Walteretal ,Tanmay S.et al and Saroj Kandel et al presented case reports where severe B12 deficiency masquerading as thrombotic thrombocytopenic purpura (TTP) like picture and can even present as multi organ dysfunction syndrome (MODS).[4,5,6]

HISTORICAL REVIEW:

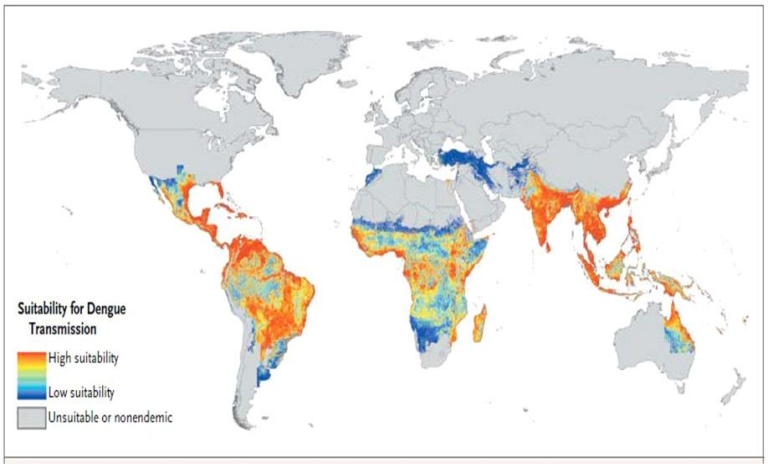
Dengue fever was also known as "Dandy fever" at one time. In a Chinese medical encyclopaedia, the first known instance of Dengue fever was documented. Due to symptoms of myalgia and arthralgia, Benjamin Rush coined the phrase "break bone fever" during the Philadelphia pandemic of 1780.[7]

During the 1950s, severe dengue fever was initially identified during outbreaks in the Philippines and Thailand. Most Latin American and Asian nations are now affected by severe dengue fever. Hemorrhages can make it worse; shock and death have been documented in outbreaks in Australia (1897), Greece (1928), and Formosa (1931). Its viral nature was proven in 1906, and its mosquito-borne transmission by Aedes aegypti was demonstrated in 1903.[8] In 1944, in Japan, Sabin isolated the virus and demonstrated the existence of its viral serotypes, but later in 1944, in Calcutta, one was isolated from a US soldier and was considered as a first report for a long time.[9]

Following WWII, the frequency of pandemics, which had enhanced transmission of several serotypes, began in Southeast Asia, resulting in dengue hemorrhagic fever epidemics.[8]

The first significant epidemic disease in India, which was clinically consistent with dengue fever, occurred in Madras in 1780 and spread throughout the nation.[9]

FIGURE 1: Areas based on suitability of dengue transmission[10]



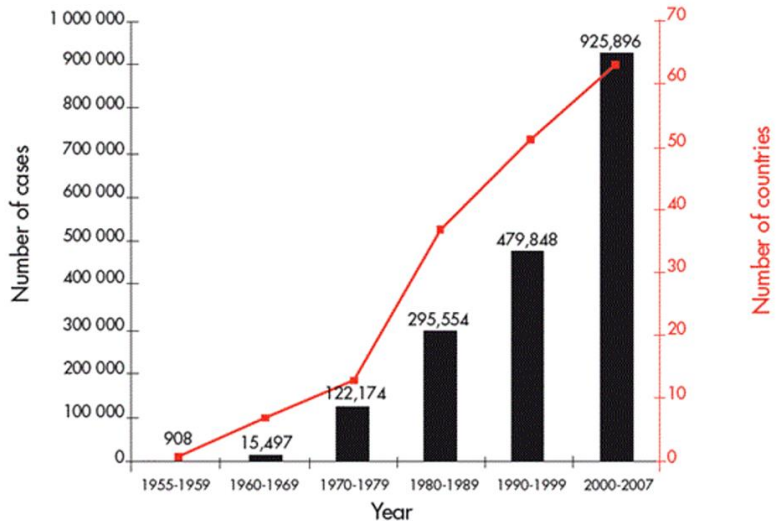


FIGURE 2: Average annual number of DF/DHF cases (WHO 1955-2007)[10]

EPIDEMIOLOGY:

DENGUE GLOBAL BURDEN

More than 2.5 billion people — 2/5th of the world's population – are at danger in tropical and subtropical nations. Dengue fever affects more than 50 million people globally each year. Every year, over 5,000 patients with DHF require hospitalisation. The most majority (about 90%) are youngsters under the age of five, with a death rate of roughly 2.5 percent.[11]

Dengue fever outbreaks are becoming more common. During epidemics, infection occurs at a rate of 40 percent to 50 percent in people who have never been exposed to the virus before,even though the risk of infection exists in 129 countries, 70% of the total actual burden lies in Asia.[12]

The number of dengue cases reported to WHO has increased over eight fold over the last two decades, from 505,430 cases in 2000, to over 2.4 million in 2010, and 5.2 million in 2019. Reported deaths between the year 2000 and 2015 increased from 960 to 4032.[12]

DENGUE BURDEN IN INDIA

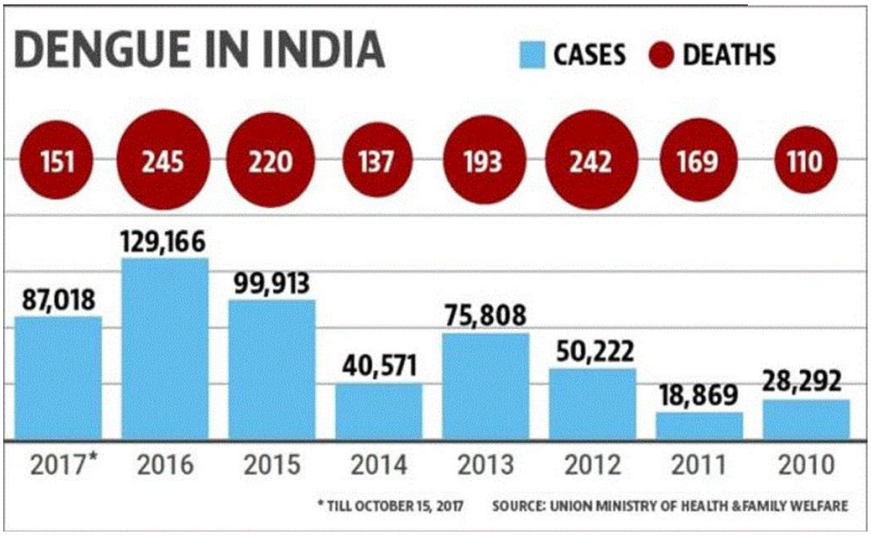


FIG 3: No. of cases and no. of deaths due to dengue in 2010-2017[13]

Major outbreaks in India occurred in 1996, 2006, and 2010, with each epidemic seeing a decrease in case fatality rates due to better management strategies used after the NVBDCP released national dengue treatment guidelines. The illness follows a seasonal pattern, peaking during and following the monsoon season. However, transmission persists in the southern and western states

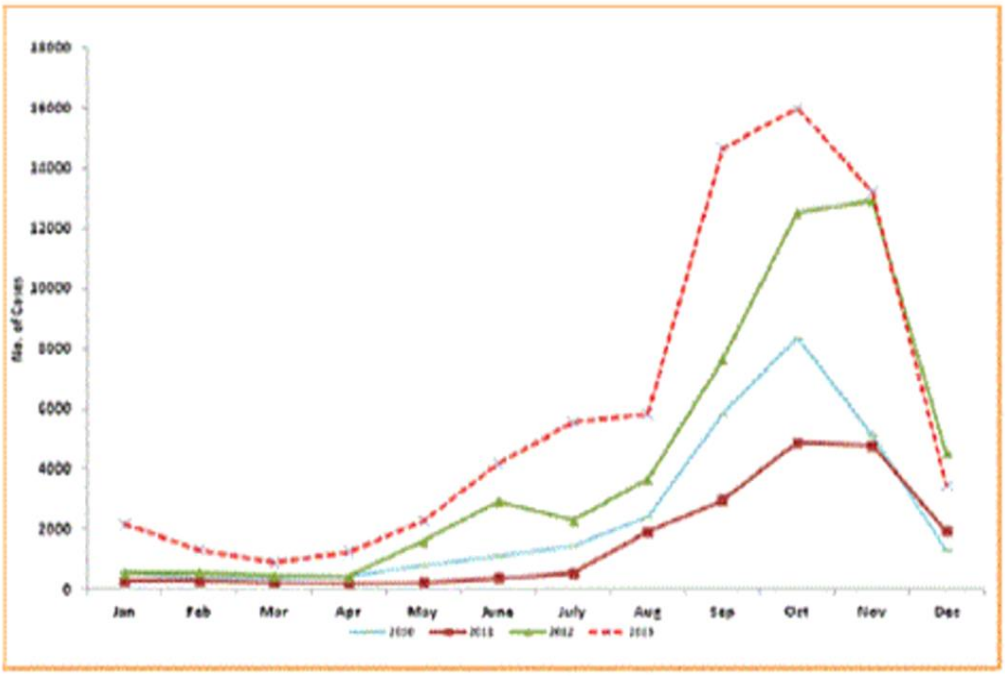


FIG 4: Seasonal pattern of Dengue

Calcutta (1963), Vishakapattanam (1964), West Bengal (1968),Ajmer (1969), Kanpur (1969),Delhi (1970), Rajasthan (1985) and Delhi (1996) are the epidemics which occurred in India till now.[9]

In India, a major DF epidemic occurred during September and October 2006, resulting in over 12000 cases and 184 fatalities, with about 3366 cases and 65 deaths occurring in Delhi alone. In 2006, the number of cases reported decreased somewhat from 2005, although the case fatality rate remained over 1%.[14] In recent years, India has recorded 101192 cases with 172 fatalities in 2018, and 157315 cases with 166 deaths in 2019. 39419 instances were recorded in 2020, with 56 fatalities.[13]

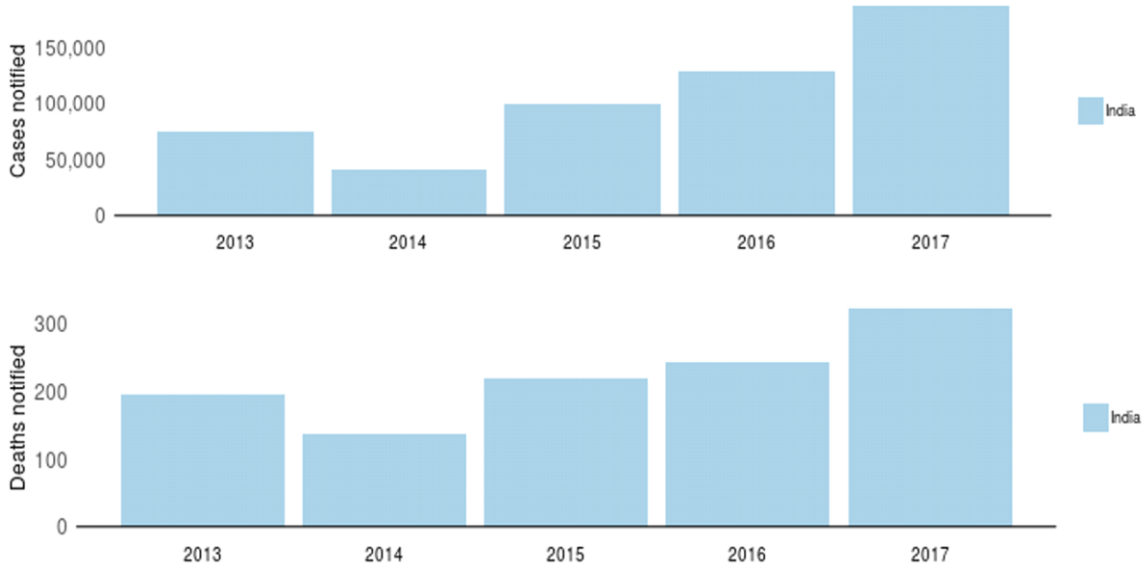


FIGURE 5: Dengue cases notified and deaths notified in India in 2013-2017[13]

THE VIRUS:

The Flavivirus genus includes the Dengue virus. There are four different types: DEN1, DEN 2, DEN 3, and DEN 4. Later, a novel serotype of dengue virus known as DEN 5 was discovered in a Malaysian 37-year-old (Sarawak state). Researchers discovered that this new serotype, DEN 5, has genetic similarities with the previous four serotypes. The NS antigen is a nonstructural protein that comes in five different forms and is encased in a lipoprotein envelope. Envelope protein's principal purpose is to attach to the host cell and to cause RBC haemagglutination. NS-1 has a direct relationship with viral titres, and it is greater in DHF patients than in dengue fever patients. The presence of high NS-1 levels in the blood suggests that the patient is at risk of having dengue hemorrhagic fever. DENV-1 is divided into three subtypes, DENV-2 into six (one of which is seen in nonhuman primates), DENV-3 into four, and DENV-4 into four. [15]

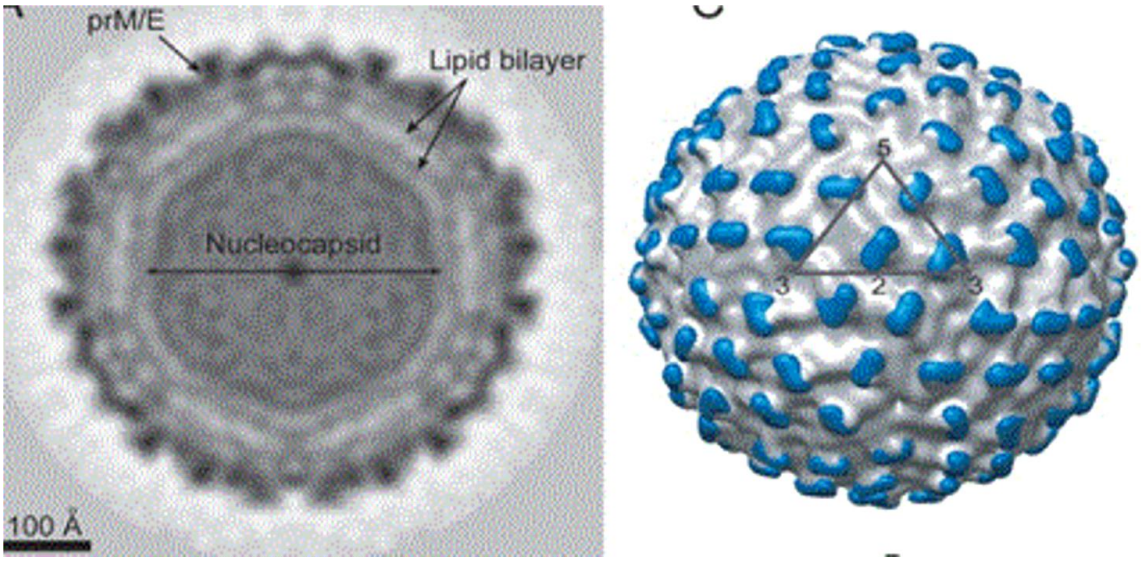


FIGURE 6: Electron microscopic picture of Dengue virus.[10]

THE VECTOR AND THE LIFE CYCLE OF DENGUE VIRUS:

Dengue virus is spread from person to person by the bite of a female Aedes mosquito. In India, Aedes aegypti is the most common vector in most metropolitan areas, however Aedes albopictus has also been implicated in several states. In some countries, other species such as Aedes polynesiensis and Aedes niveus have been identified as secondary vectors. [11.] Aedes aegypti has Aedes is a biter who bites during the day.

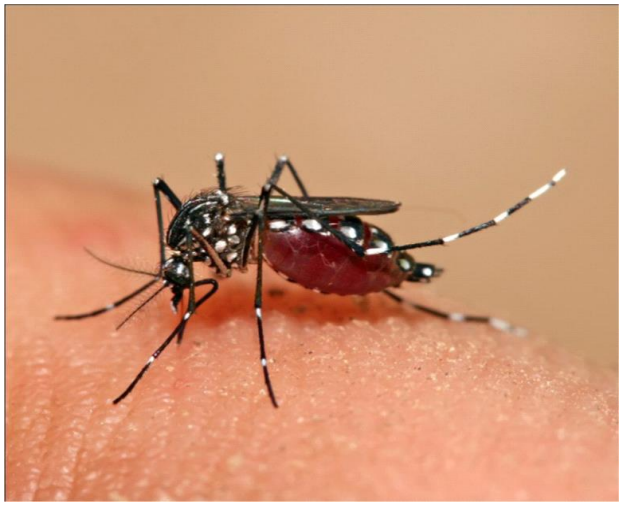


FIGURE 7: *Aedes aegypti mosquito.*[10]

The main offender, Aedes aegypti, may be found in water storage containers, unused tyres, water reservoirs, overhead tanks, unused grinding stones, coconut shells, disposable cups, and other places. Tree holes, bamboo stumps, latex collecting cups in rubber trees, coconut shells, and other natural habitats are preferred by Aedes albopictus. Aedes albopictus has been seen reproducing in residential settings.

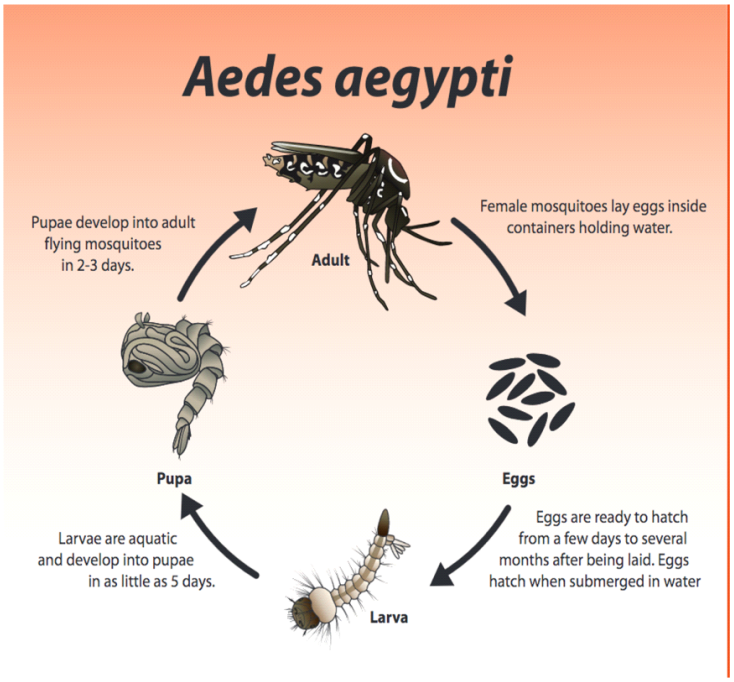


FIGURE 8: Life cycle of Aedes aegypti[11]

The Aedes mosquito is a tropical and subtropical species that is found all over the world. Aedes aegypti also has a hard time surviving over 1000 metres owing to the lower temperatures. Aedes aegypti is a jittery feeder and a discordant species that is highly tamed and anthropophilic. Aedes albopictus is an aggressive feeder that feeds on both humans and animals. It does not require a second blood meal to complete its gonotropic cycle. When a dengue-infected individual is bitten by an adult female mosquito, the virus enters the insect and multiplies. Extrinsic incubation time is a period of 8-12 days during which the virus replicates.

THE HOST:

The virus's primary amplifying host is humans. Human viraemia peaks 2 days before the beginning of the fever (non-febrile phase) and lasts 5–7 days after the onset of the fever (febrile phase). Vector species become infected while feeding on the viremic human during these periods of sickness. The bite of a vector is the primary mode of transmission. There have also been instances of congenital dengue infections in newborns delivered to women who were infected late in pregnancy.

The dengue virus enters the body through the skin as a mosquito feeds on blood. The incubation time for intrinsic infections is typically 4-10 days, followed by a wide range of symptoms, however most infections are asymptomatic or subclinical. The virus is present in blood during the acute phase, and its elimination usually corresponds with defervescence. Serotype-specific and cross-reactive antibodies, as well as CD4+ and CD8+ T cells, remain detectable and quantifiable in the human host for years after infection.

Any serotype that is initially infects confers lifelong immunity to that serotype. The severe form of dengue (DHF/DSS) is caused by secondary infection with another serotype or several infections with different serotypes at the same time.

Serotype 1 infection followed by Serotype 2 infection is more hazardous than Serotype 4 infection followed by Serotype 2. Antibody-dependent enhancement (ADE) of infection has been postulated as a mechanism to explain severe dengue fever in both secondary and initial infections in babies. Non-neutralizing, cross-reactive antibodies generated during a primary infection or acquired passively at birth bind to epitopes on the surface of a heterologous infecting virus,

allowing the virus to enter host cells with Fc receptors . Cross-reactive memory T cells are rapidly activated after a subsequent infection, proliferating and releasing cytokines.

TRANSMISSION CYCLE: [11]

Enzootic cycle:This cycle persists between Aedes and monkeys, with viruses causing no disease and viraemia lasting only 2–3 days in monkeys. Monkeys have shown evidence of serological isolate for all four dengue serotypes (DEN 1 to 4).

Epizootic cycle This occurs as a result of bridge vectors connecting adjacent human epidemic cycles. During the years 1986–1987 at a research region in Sri Lanka, the epizootic cycle was first seen among touqe macaques (Macaca sinica). On a serological basis, 94 percent of macaques within 3 kilometres of the research region were confirmed to be infected.

Epidemic cycle : The human-Aedes aegypti-human cycle is maintained by recurrent or cyclical epidemics. All serotypes circulate, resulting in hyperendemicity. Aedes aegypti is an effective vector due to its strong anthrophilicity, numerous feeding behaviour, and highly domesticated environments. As a result, the survival of dengue virus is contingent on the establishment of large viral titres in human hosts in order to continue mosquito transmission. A large vector population develops up in man-made storage containers in desert zones when rainfall is scarce during the dry season.

A variety of factors have a role in the onset and continuation of an epidemic, including:

THE VIRUS

In humans, the virus strain has an impact on the severity and duration of viraemia.

THE VECTOR

The density, behaviour and vectorial capacity of vector population.

THE HOST

The human population's susceptibility (influenced by both genetic factors and pre-existing immune profile).

PATHOGENESIS:

The immunological responses of the host are important in the pathogenesis of Dengue, and cytokine storm is the most common mechanism. These cytokines are important in the progression of bleeding and shock.

Coagulopathy[1]

The cause of coagulopathy in those who have dengue fever is unknown. The risk of bleeding is increased when thrombocytopenia is combined with coagulopathy. Coagulopathy can also be caused by the release of heparin sulphate or chondroitin sulphate from the glycocalyx.

The levels of tumour necrosis factor receptor (TNF), interferon-gamma (IFN-gamma), and interleukin-2 (IL-2) in the blood are elevated in shock. C1q, C3, C4, C5–C8, and C3 pro-activators all decreases, whereas C3 catabolic rates increase. Through the nitric oxide final route, these substances may combine to enhance vascular permeability. The levels of factor XII are low. DIC (disseminated intravascular coagulation), liver injury, and thrombocytopenia all work together to worsen the scenario.

Capillary leakage and Shock[1] Hypotension - Plasma leakage occurs as a result of a transient change in the endothelium fibre matrix's properties. Anti-NS1 antibodies act as autoantibodies that cross react with platelets and non-infected endothelial cells, causing capillary permeability to be disrupted. Plasma leakage can present as a variety of symptoms, including haemoconcentration, ascites, and pleural effusion. It generally appears 3 to 7 days after the onset of the disease. [16]

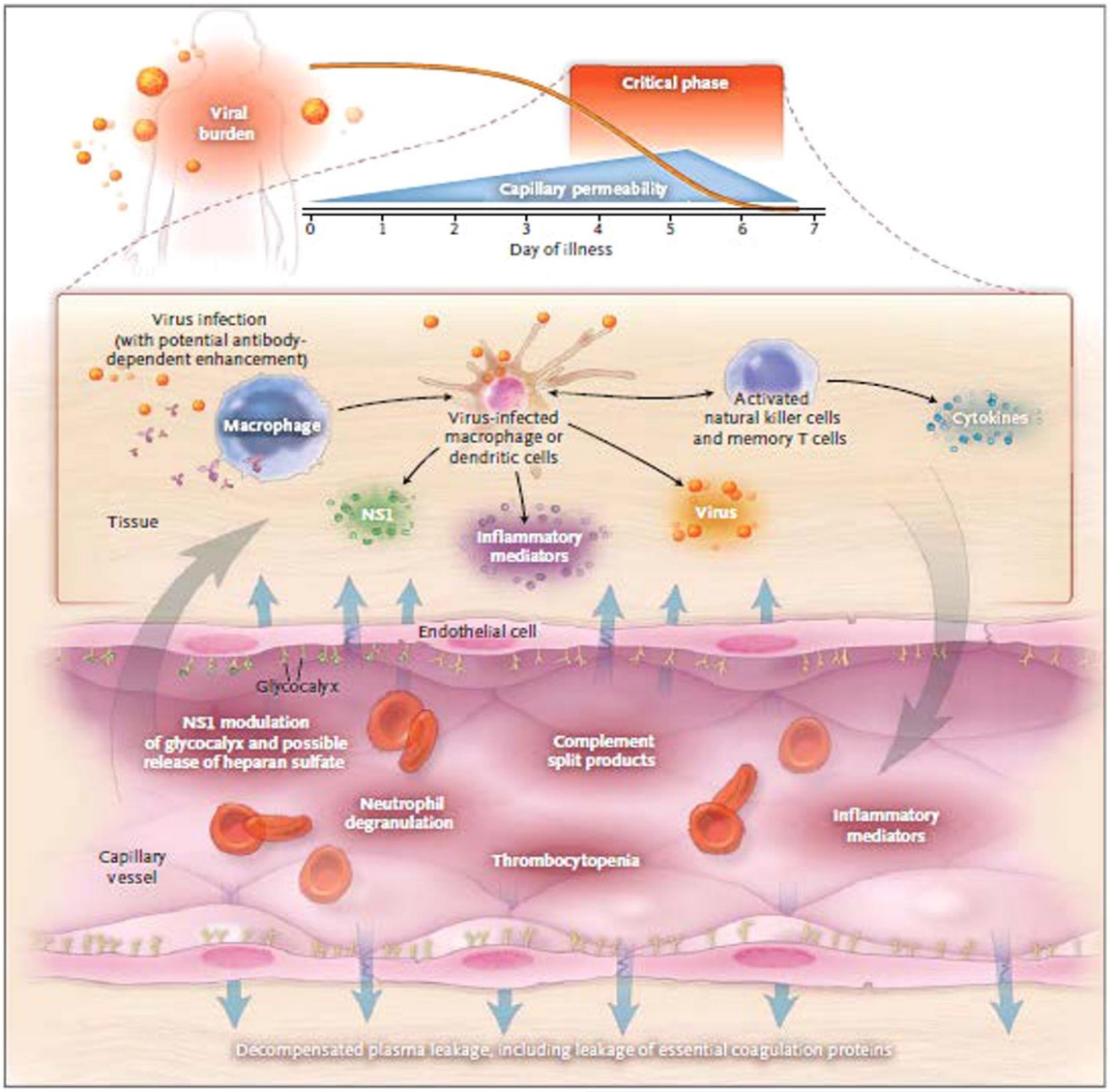


FIGURE 9*:* Pathogenesis in dengue infection[17]Reproduced with permission from (scientific reference citation), Copyright Massachusetts Medical Society.

Perivascular oedema can be seen in the soft tissues, and extensive diapedesis of red cells can be seen on the vessel walls.

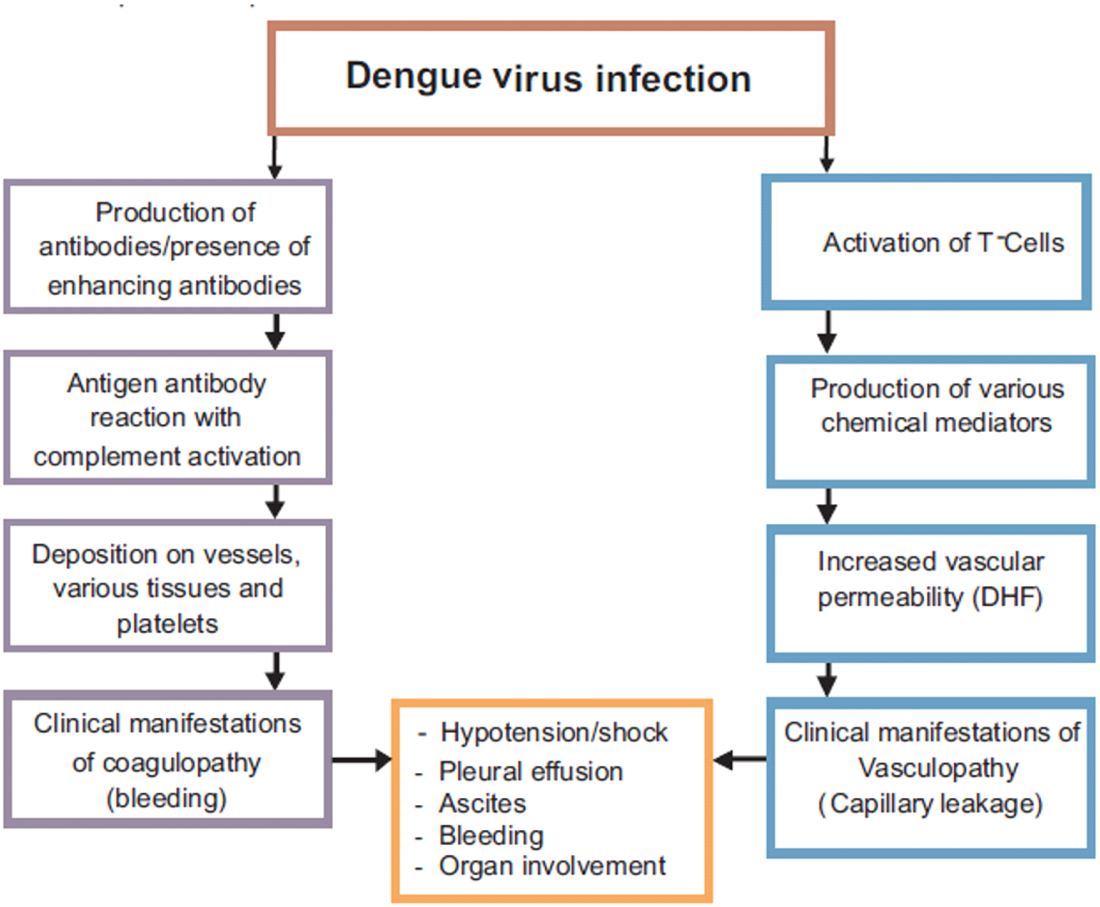


FIGURE 10*:* Sequence of changes in Pathogenesis of dengue infection [1]

CLINICAL FEATURES:

A person infected with the Dengue virus can be asymptomatic or symptomatic, with symptoms ranging from moderate undifferentiated fever to severe bleeding and shock syndrome. The clinical manifestations are influenced by a number of factors, including age, the immunological state of the host virus strain, and whether the infection is primary or secondary. Various non-

specific constitutional symptoms such as fever, headache, backache, and widespread malaise may occur after an average intrinsic incubation time of about 4–6 days (range of 3–14 days). Retroorbital discomfort on eyeball pressure or eye movement, photophobia, muscular and joint aches, and backache are all possible after that. These symptoms might last anywhere from a few days to a few weeks. It's worth mentioning that the frequency and intensity of these signs and symptoms vary depending on the severity of Dengue fever.

Fever: The core body temperature is generally between 39 and 40 degrees Celsius, and it can be biphasic, lasting 5–7 days in most cases.

Rash: During the first 2-3 days, a transient broad rash appears on the head, limbs, back, and chest area. The rashes go away by the end of the febrile period, however some patients may have itchiness.

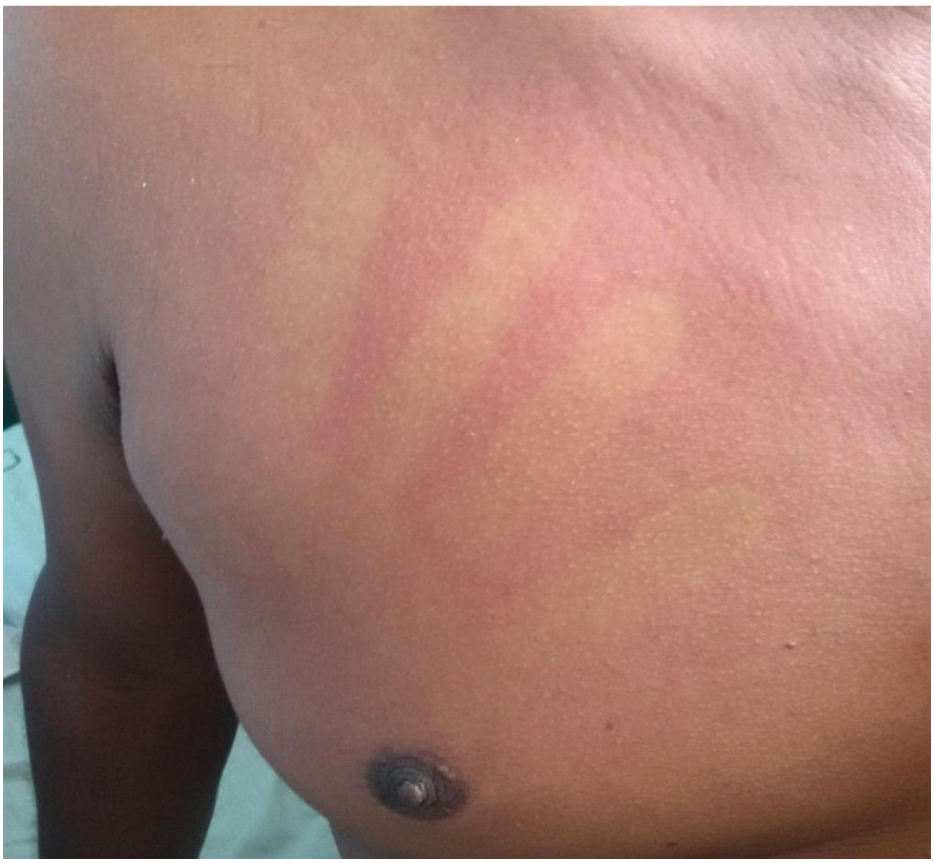


FIGURE 11: Impression mark on skin of a dengue patient. [1]

Haemorrhagic manifestations:

A positive tourniquet test and/or petechiae may be used to indicate skin haemorrhages. In severe DF exacerbated by thrombocytopenia, further bleeding symptoms such as epistaxis, hypermenorrhea, and gastrointestinal bleeding might develop.

Tourniquet test:[11]

A blood pressure cuff is inflated halfway between the systolic and diastolic readings. If there are more than 10 petechiae per square inch, the test is positive. In DHF, however, it is greater than 20. During severe shock, the test may only be slightly positive or negative (DSS).

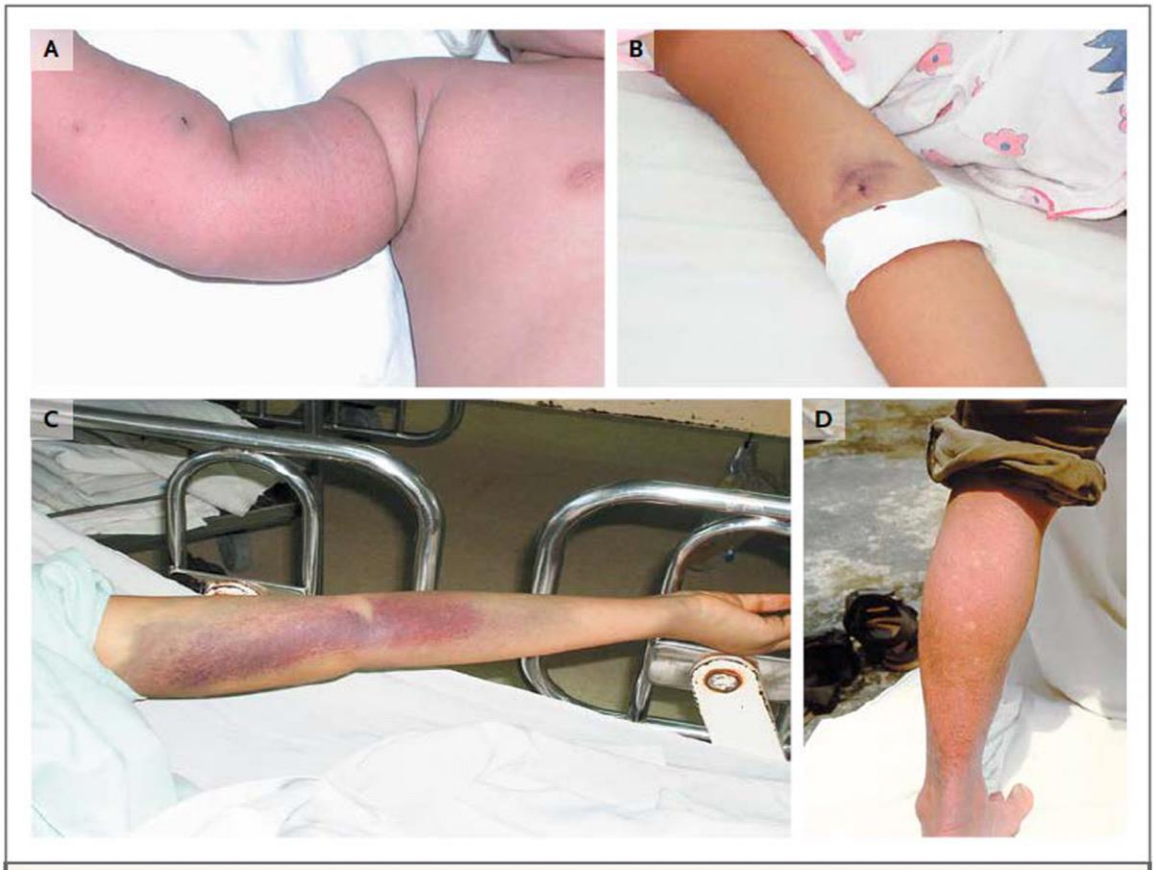


FIGURE 12: Hemorrhagic manifestations of dengue infections. [17]

CLINICAL CRITERIA FOR DF / DHF/DSS[1,10] :

Dengue Fever (DF):

- An acute febrile sickness lasting 2-7 days with 2 or more of the symptoms listed below:

  Headache, discomfort behind the eyes, bodyache, joint pain, skin rash, and bleeding tendencies are only a few of the symptoms.

DHF (Dengue Haemorrhagic Fever): A case that meets the clinical criteria for dengue fever.

Plus

- Haemorrhagic tendencies are indicated by one or more of the following features:

i. In a tourniquet test, there were more than 20 petechial spots.

ii. gastrointestinal (GI) and mucosal haemorrhage

iii. Petechial rashes

Plus

- Low platelet count (less than 100 000 cells per cubic millimetre)

Plus

- One of the following is a manifestation of the third space fluid collection:

i. A 20% or more increase in average hematocrit for a certain age and sex,

ii. After fluid treatment, the hematocrit drops by more than 20%.

iii. Evidence of fluid accumulation in the third space, either clinically or radiologically.

Dengue Shock Syndrome:

The features of DHF are listed in their criteria, combined with signs of circulatory shock (such as tachycardia and a pulse pressure of less than 20 mm Hg).

EXPANDED DENGUE SYNDROME (EDS) [1,10]:

In DF or DHF, organ involvement might be mild or severe. Comorbidities and other coinfections are frequently linked with unusual DF/DHF symptoms.

The following are the clinical symptoms of EDS:

1 NEUROLOGICAL:

Encephalopathy, febrile seizures, encephalitis, and intracranial haemorrhage are all symptoms of encephalopathy.

2. HEPATIC OR GASTROINTESTINAL:

Hepatitis Acute, fulminant hepatic failure Cholecystitis, Cholangitis, Acute Pancreatitis

3. RENAL :

Acute renal failure, Acute tubular necrosis, Hemolytic uremic syndrome(HUS)

4. INVOLVEMENT OF THE CARDIAC SYSTEM:

Cardiac arrhythmia, myocarditis, cardiomyopathy, and pericardial effusion are all conditions that can occur in the heart.

5. RESPIRATORY:

Pulmonary edema, Pleural effusion, ARDS, Pulmonary haemorrhage,

6. EYE INVOLVEMENT:

Conjunctival bleed,Optic neuritis, Macular haemorrhage, Visual impairment

Natural course of dengue Infection:[1,10]

The clinical course of disease is divided into three stages:

- 1.Febrile phase
- 2.Critical phase
- 3.Convalescent phase

1. **Febrile phase**

- Patients have a high-grade fever that appears suddenly and lasts for 2-7 days.
- Facial flushing, generalised body pain, rash, vomiting, and headache are some of the symptoms.

- Sore throat, conjunctival injection, and pharyngeal injection can also accompany.

- The earliest test data reveals a decrease in leucocytes, followed by a decrease in platelet count.

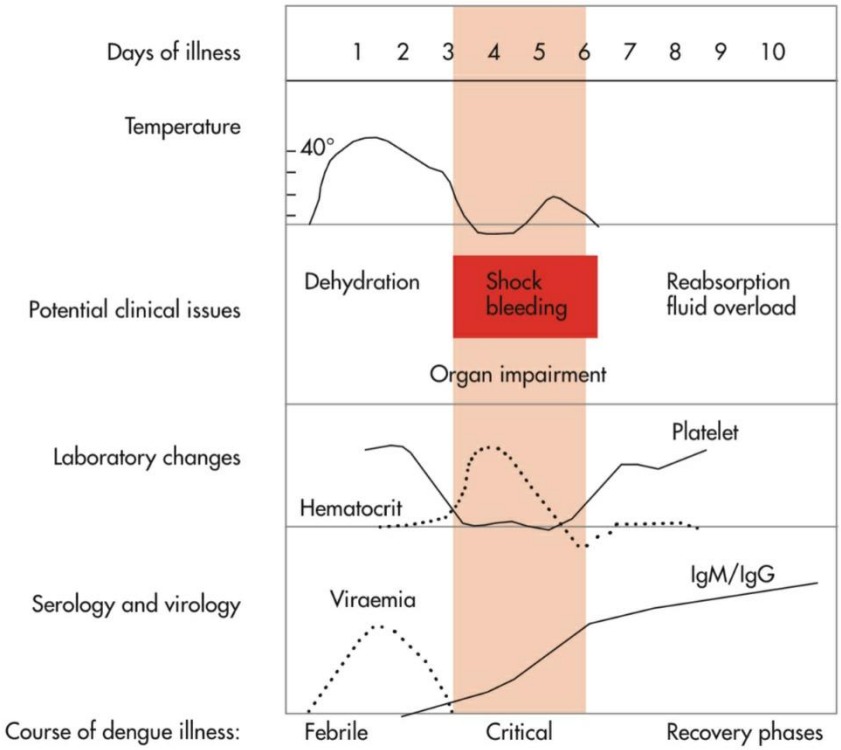


FIGURE 13: Natural course of dengue illness[10]

2. Critical phase (Leakage phase)

- The critical phase usually begins after the third day of fever (although can start sooner) or around defervescence, which is marked by a fast drop in temperature.
- In other viral infections, the patient's health improves as the fever drops; but, in severe dengue infection, the patient's condition might deteriorate, resulting in third-space plasma leakage or organ failure.
- The plasma leaking phase lasts 36-48 hours on average.

3. Convalescent phase (recovery phase)

- Plasma leakage ceases, and fluids previously accumulated in third spaces such as the peritoneum and pleural cavity are redistributed, resulting in widespread pruritus.
- Typically, the recovery of a low platelet count is preceded by the recovery of a low white blood cell count.
- This phase generally occurs after a fever has been present for 6-7 days and lasts for 2-3 days.

If fluid replacement is not done carefully, the patient may develop pulmonary edema as a result of fluid excess.

CASE DEFINITION:[1,10]

- Probable DF/DHF:

a. During an outbreak of dengue fever, a case that matches the clinical criteria (Clinical Criteria).

OR

b. NS1 antigen or IgM positive but not by ELISA.

(A positive test done by RDT will be considered as probable due to relatively less sensitivity and Specificity)

- Dengue Fever Confirmed:

- A case with symptoms that meets clinical criteria for dengue fever and includes at least one of the following :

a. Dengue virus isolation (culture positive) from serum, plasma, or leucocytes.

b. ELISA positive demonstration of IgM antibody titer in a single serum sample.

c. Positive NS1-ELISA result in the serum.

d. After 2 weeks of sickness, if IgG sero-conversion is seen in matched sera, with a fourfold rise in IgG titer.

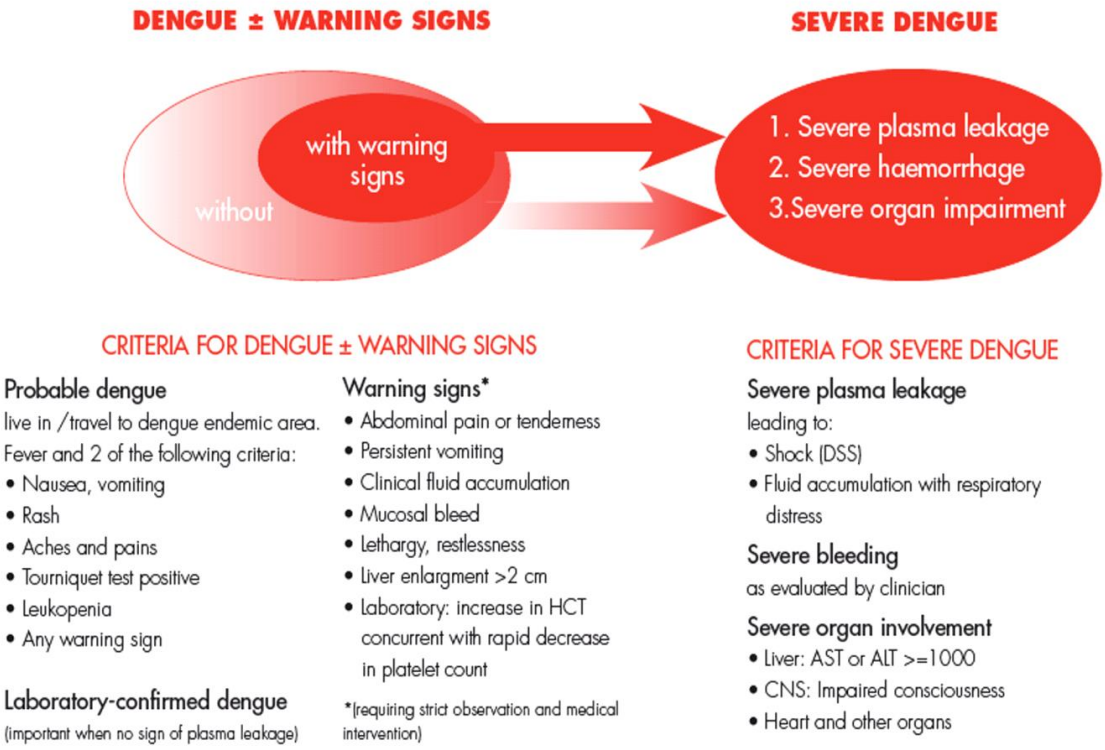


FIGURE 14: Criteria for dengue infections.[10]

GRADING OF DF/DHF[1,10]:

Dengue Fever (DF):

- 2-7 day pyrexia (fever) with 2 of the following clinical features: Headache, bodily discomfort, pain behind the eyes, and joint pain are all common symptoms.
- With or without thrombocytopenia, leucopenia, and no evidence of plasma leakage in the laboratory

.

Dengue Haemorrhagic Fever (DHF I):

- With a positive tourniquet test and evidence of plasma leakage, the patient meets the criteria for dengue fever.
- Thrombocytopenia with a platelet count of 1,00,000/cu.mm and a hematocrit increase of above 20% from baseline.

Dengue Haemorrhagic Fever II (DHFII):

- Above DHF I criteria, as well as stomach discomfort and signs of spontaneous bleeding in the skin or other organs (epistaxis, bleeding gums, black tarry stool).
- Thrombocytopenia (platelet count 100000/cu.mm) and Hct levels increase by more than 20% from baseline.

Dengue Haemorrhagic Fever III (DHFIII / DSS):

- Thrombocytopenia with platelet count 100000/ cu.mm and Hematocrit rise of more than 20% above baseline, as well as clinical evidence of circulatory failure (tachycardia, cold peripheries, narrowing of pulse pressure)

- Thrombocytopenia with platelet count 100000/ cu.mm and Hematocrit rise of more than 20% above baseline

Dengue Haemorrhagic Fever IV (DHFIV / DSS):

- Profound shock along with undetectable blood pressure or pulse.

- Thrombocytopenia with platelet count <100000/ cu.mm and Hematocrit rise more than 20% above baseline.

LABORATORY INVESTIGATIONS:[1,10]

Initial symptoms of dengue fever in endemic regions might be confused with those of other common diseases such as chikungunya, malaria, urinary tract infection, viral infection, leptospirosis, and typhoid. As a result, excluding such situations is critical for appropriate dengue fever therapy.

ELISA-based NS1 antigen tests: Dengue NS1 antigen is a highly conserved glycoprotein that is generated in both membrane-associated and secretory forms, and it is prevalent in the serum of dengue patients in the early stages of infection. It has been shown to be effective in diagnosing acute dengue infections. A simple test with good sensitivity and specificity. The NS1 antigen has allowed for early identification of patients in the viremic stage, which has epidemiological implications for limiting disease transmission. For DENV, a commercially accessible NS1 ELISA-based antigen test is available, and numerous researchers have reviewed it and praised it for its sensitivity and specificity**.** Because of its great specificity, this NS1 test might be beneficial for differentiating amongst flaviviruses. For the first five days of sickness, it is typically positive.

IgM Antibody Capture Enzyme-Linked Immuno Sorbent Assay (MAC-ELISA):

In recent years, MAC-ELISA has become increasingly popular. A simple test that necessitates the use of extremely basic equipment. Based on collecting dengue specific antibodies in the serum using anti-human IgM antibodies that have previously been linked to the solid phase. A particular colour reaction is also introduced as an enzyme substrate for simple identification.

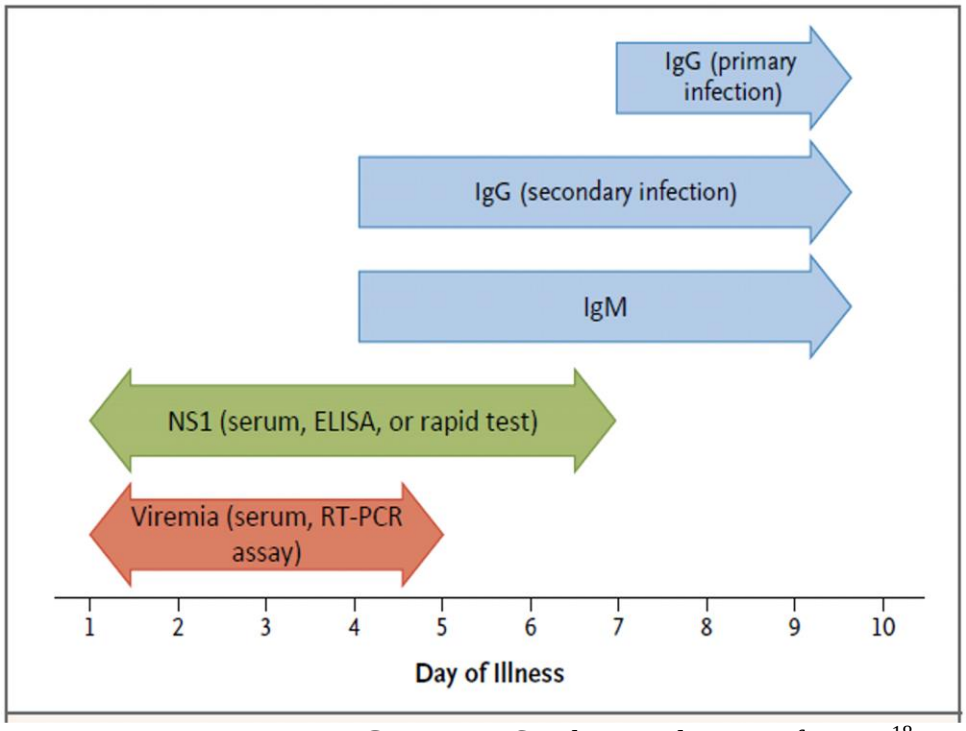


FIGURE 15: Serology in dengue infection.[18]

Anti-dengue IgM antibodies develop more quickly than IgG antibodies and are generally detected by the fifth day of sickness. However, the rate of recovery varies greatly across people. IgM antibodies are detected on days 2-4 following the beginning of sickness in some people, but not in others, even after 7-8 days. IgM may remain for up to 90 days in certain initial infections, although it is usually undetectable after 60 days. As a result, it has become a significant tool for DF/DHF surveillance. It may be used for clinical monitoring for viral disease and population-based sero-surveys in non-endemic regions, with the assurance that any positives indicate recent infections. It's especially helpful for hospitalised patients who are admitted at the end of their illness, when measurable IgM levels are already present in their blood.

Polymerase chain reaction (PCR): RT-PCR (reverse transcription polymerase chain reaction)-based molecular diagnostics, such as one-step or nested RT-PCR, real-time RTPCR, or nucleic

acid sequence-based amplification (NASBA). They have gradually supplanted previous viral isolation methods as the current standard approach for detecting dengue virus.

IgG-ELISA: A haemagglutination inhibition (HI) test has been developed and compared to an IgG-ELISA. It's a good way to tell the difference between primary and secondary dengue illnesses. It is simple and straight forward to use, however it only detects previous illnesses. As a result, it isn't regarded a diagnostic test.

Apart from MAC-ELISA and IgG-ELISA, there are a few serological assays for diagnosing dengue infection, including complement fixation (CF), neutralisation test (NT), and hemagglutination-inhibition (HI). However, due to a variety of technological difficulties, they are not commonly utilised.

HIGHLY SUGGESTIVE (if one of the following)	**CONFIRMED** (if one of the following)
	1) PCR positive
	2) Virus Culture positive
1)IgM (+) in a single serum sample	3) IgM seroconversion in a paired sera
2) IgG (+) in a single serum sample with a high titre of 1280 or higher	4) IgG seroconversion in paired sera , or four fold IgG titer increase in paired sera

Table 1 – Diagnosis of Dengue Fever

RDTs (Rapid diagnostic tests):

At times, these tests may produce a misleading negative result. The usefulness of such tests in guiding clinical therapy of DF is debatable. In clinically questionable patients, a negative RDT test does not rule out dengue infection.

Sample collection:

The collection, storage, processing, and transportation of materials are all critical for accurate dengue diagnosis. The following information is included in the samples sent to the lab for confirmation: The laboratory will use the date of beginning of fever and the date of sample collection to determine the kind of test to perform (NS1 for samples obtained from day 1 to day 5, and IgM after day 5).

The laboratory findings of acute DF:

- During the febrile phase, leucopenia with a declining trend in neutrophils is noticed.

  Platelet counts are typically within normal ranges.

- Platelet count reductions of 1 to 1.5 lakh cells/mm3 are frequent.

- Around half of all DF patients have a platelet count of less than one lakh cells per millilitre, although severe thrombocytopenia with a count of less than 0.5 lakh cells per millilitre is uncommon.

- A little increase in Hematocrit of about 10%.

The laboratory findings of DHF:

- In the early stages of a fever, the WBC count may be normal, but there may be a high concentration of neutrophils. Following that, towards the conclusion of the febrile period, there is a decrease in WBCs and neutrophils. The ratio of neutrophils to lymphocytes (N:P) (neutrophils :lymphocytes) and the change in total leucocyte count (5000 cells/mm3) are effective in predicting plasma leakage during the crucial phase of dengue sickness.

- By the conclusion of the febrile phase, but before the start of shock or the end of the afebrile phase, the platelet count has dropped to 100 000. The severity of DHF is directly proportional to platelet count.

- Shortly after the platelet count drops, the hematocrit rises abruptly. Haemoconcentration, or a 20 percent increase in hematocrit from baseline.

- Thrombocytopenia (1 lakh cells/mm3) is a condition in which the blood platelets are abnormally low. Almost all instances of DHF, especially those involving shock, have an increase in hematocrit.

- Early volume replacement and bleeding symptoms can sometimes influence the hematocrit level. In rare situations, an elevated AST/ALT ratio (more than 2) may be seen.

Abnormalities in electrolytes: Sodium and calcium levels in the blood are mildly reduced.

COMPLICATIONS OF SEVERE DENGUE:

Liver failure : Hepatocytes and Kupffer cells in the liver aid viral propagation. Patients with severe DHF have considerably greater levels of liver enzymes aspartate transaminase and alanine transaminase, and plasma proteins, notably albumin, are significantly lower.

Hepatitis can also cause fulminant liver failure, with increasing liver failure eventually leading to hepatic encephalopathy. There may or may not be jaundice present.

CNS Complications : Neurological examination may reveal hyper-reflexia and extensor plantar response if the CNS is involved (hepatic encephalopathy).

Hepatic dysfunction, electrolyte imbalances, cerebral edoema (due to vascular changes leading to extravasation of fluid), hypoperfusion (due to shock) are all factors that contribute to the development of encephalopathy. In patients with symptoms of encephalitis, the virus has also been isolated from the cerebrospinal fluid (CSF).

Cardiac complications: Asymptomatic bradycardia to life-threatening illnesses such as pericardial effusion and myocarditis. The majority of them are reversible when combined with other symptoms of dengue fever.

MANAGEMENT:

Management of dengue Fever (DF) [16,19] :

Dengue fever is generally treated with symptomatic and supportive treatment. When the acute phase is still present, bed rest is recommended.

To lower the temperature below 38.5°C, use cold tepid sponging.

Antipyretics (paracetamol) can be used to lower the body's core temperature. Because NSAIDS such as Ibuprofen and Aspirin can induce gastritis, vomiting, platelet malfunction, severe bleeding, and acidosis, they should be avoided. Depending on the severity of the fever and body discomfort, the paracetamol dose can be repeated every 6 hours.

Excessive sweating or vomiting should be treated with oral fluids and electrolyte treatment.

After the febrile phase has ended, patients should be closely watched for 24 to 48 hours to check for any warning symptoms or problems.

Management during Febrile Phase:

To lower the body temperature below 39°C, paracetamol is advised in a similar manner. Adequate oral fluids should be recommended, with fruit juices or oral rehydration solution (ORS) being preferred over plain water for the treatment of diarrheal illnesses. If the patient has diarrhoea, recurrent vomiting, or refuses to eat, intravenous hydration should be begun. Patients should be watched for both warning symptoms and indicators of shock at the same time. The

shift from febrile to afebrile is critical, and it generally happens after the third day of sickness. Serial hematocrits, which indicate hydration status, the degree of plasma leak, and the requirement for intravenous fluid therapy, may be crucial in guiding treatment decisions. Hematocrit should be measured on a regular basis, especially after the third day and over the next 1-2 days if the temperature stays normal.

Management of DHF Grade I and II: [1]

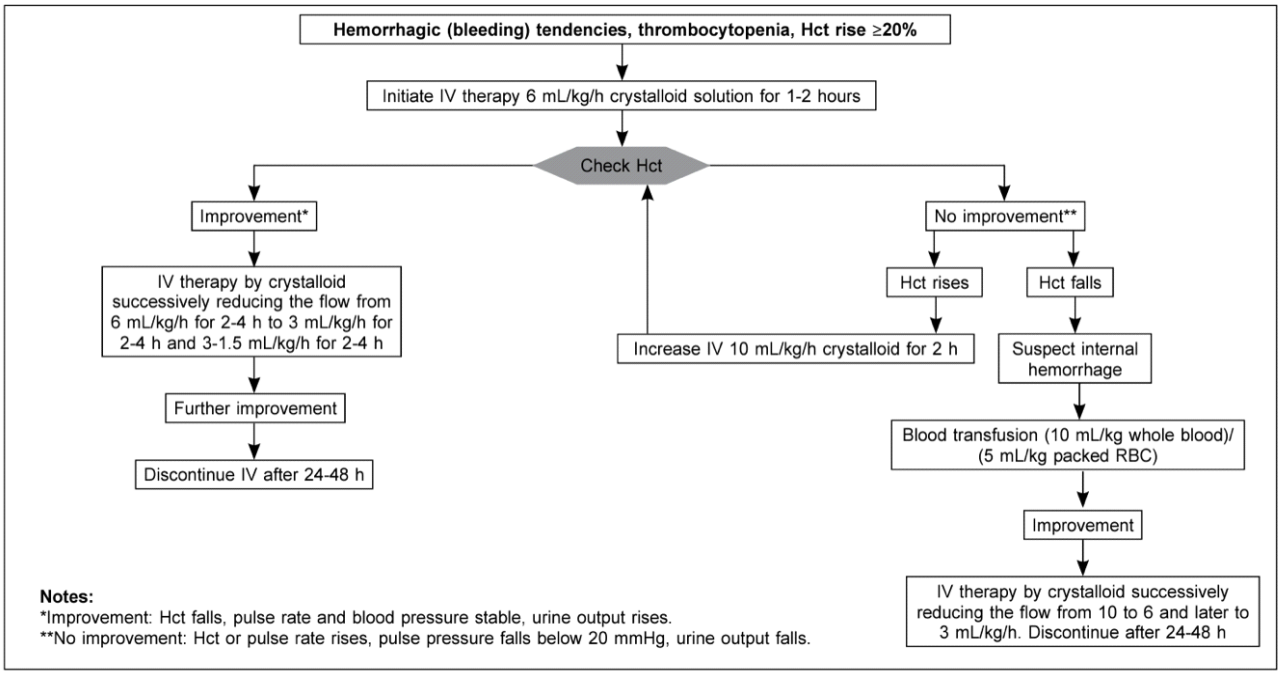
Patients with a low platelet count, a high hematocrit, or warning symptoms of dengue fever should be admitted to the hospital. To avoid additional problems, all of these people should be constantly followed. The shift from febrile to afebrile phase, which generally happens after the third day, is when a patient may suffer shock during the crucial period of sickness. If the patient experiences a drop in blood pressure, a decrease in urine output, or any other signs or symptoms of shock, the care for Grade III/IV DHF/DSS should be continued. Oral rehydration, as well as antipyretics such as paracetamol, sponging, and other measures, should be administered.

Management of Shock in DHF Grade III / IV: [16,19]

Hematocrit, platelet count, input output charting, and vital sign monitoring should all be done immediately following admission to the institution.

Treatment algorithm for DHF (Grades III and IV) is given.

FIGURE 16: Volume replacement algorithm for patients with DHF I and II [13]



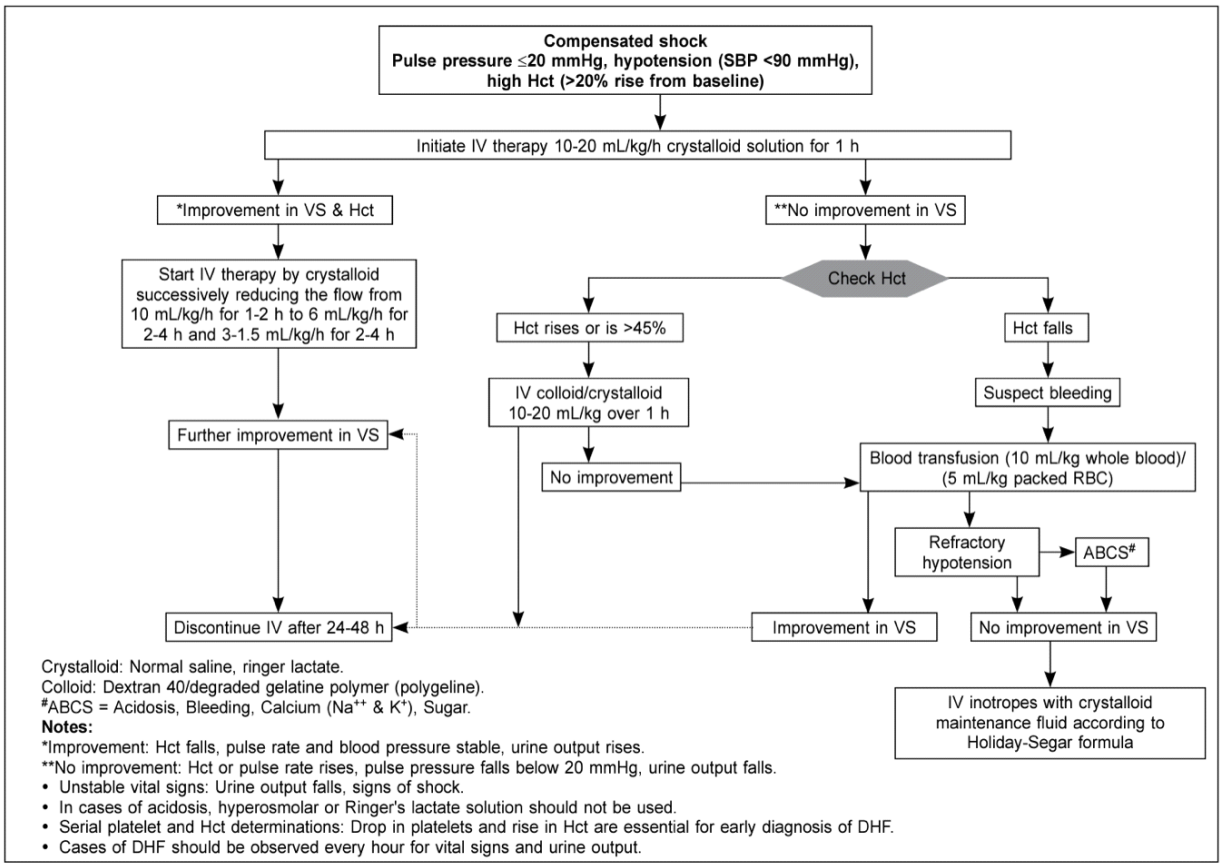


FIGURE 17: Volume replacement algorithm for patients with DHF III [19]

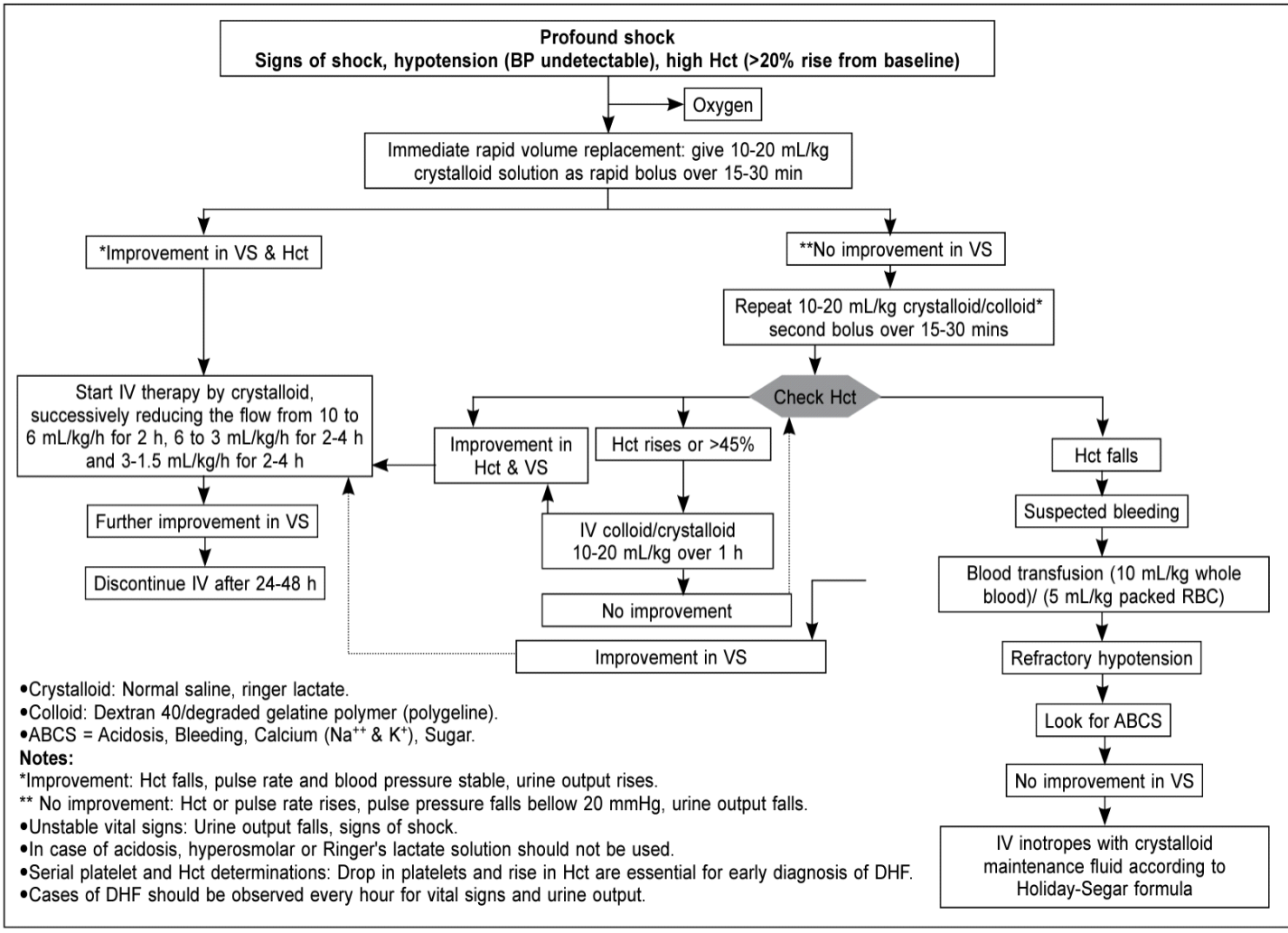


FIGURE 18: Volume replacement algorithm for patients with DHF IV [19]

Indications for Platelet transfusion: [1,16]

- Prophylactic platelet transfusion is defined as a platelet count of 10,000 cells/cu.mm in the absence of bleeding symptoms.
- Thrombocytopenia with or without haemorrhage. In individuals who present with significant bleeding, a red packed cell transfusion as well as FFP and platelets may be necessary.

Criteria for admission of a patient[16] :

- High-grade fever that persists after antipyretic treatment;
- severe thrombocytopenia and increasing hematocrit;
- patients with substantial bleeding from any cause
- Hypotension symptoms, shock, and organ involvement

Clinical or radiological evidence of plasma leakage

Criteria for discharge of patients [16,19]:

- Platelet count > 0.5 lakh cells/cu.mm No respiratory pain if afebrile for at least 24 hours without antipyretics

- Good urine production and a return of appetite

- After recovery from the shock, should wait for at least 2 to 3 days.

Identifying individuals with fluid overload

Face puffiness, dyspnea and wheezing, and abdominal distension are some of the indications and symptoms of this complication in dengue fever therapy.

Vaccine for dengue infection[20,21]

DENV vaccines are divided into five categories: live attenuated vaccinations, inactivated vaccines, viral vectored vaccines, recombinant subunit vaccines, and DNA vaccines. Sanofi Pasteur produced the first dengue vaccine, Dengvaxia® (CYD-TDV) (Chimeric Yellow fever virus-Dengue virus Tetravalent Dengue Vaccine), which was authorised by regulatory authorities in 20 countries in December 2015. The findings of an additional investigation to retrospectively identify serostatus at the time of immunisation were revealed in November of 2017. It was discovered that individuals who were inferred to be seronegative at the time of initial vaccination had a greater risk of more severe dengue sickness and dengue hospitalizations than those who were not immunised. Currently, the vaccine is intended for those aged 9 to 45 who live in endemic regions and have had at least one confirmed dengue virus infection in the past..

Vitamin B12

Vitamin B12 is a water-soluble important vitamin that is necessary for the formation of blood cells, proper neurological function, regulating B12-dependent activities, and maintaining a healthy gastrointestinal mucosa [22.] It is the only water-soluble vitamin that is sufficiently stored by the human body, with the liver serving as the major storage location, storing more than 1.5 mg of the vitamin[23.] Because vitamin B12 turnover is about 0.1 percent each day, even when a diet is completely deficient in the vitamin, insufficiency can take anywhere from 2 to 10 years to occur [23,24.]

Vitamin B12 refers to a family of complex molecules with a central cobalt atom surrounded by 4 reduced pyrrole rings, which is referred to as cobalamin. The form of cobalamin or B12 depends on the upper axial ligand bond which is linked to the cobalt atom [22,25] . While there are 5 forms of B12 (methylcobalamin, cyanocobalamin, hydroxycobalamin, aquocobalamin, and adenosylcobalamin[22,25]Methylcobalamin and adenosylcobalamin are the only two forms of B12 which are used for metabolic processes in the human body.

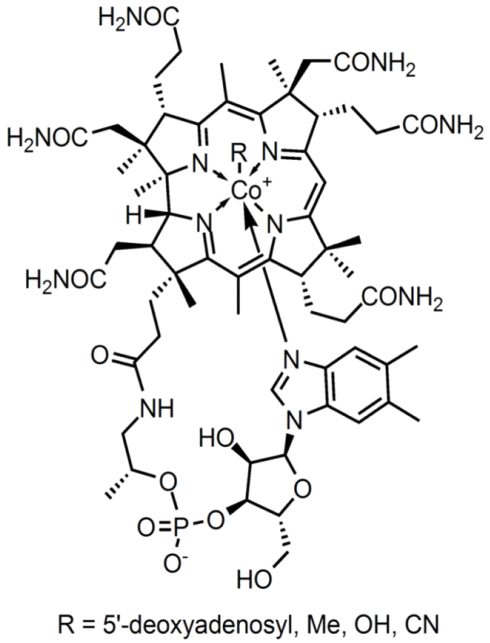


Fig 19: Vitamin B12 transport proteins

There are four known proteins at present which are involved in the absorption and transport of vitamin B12:

These are Intrinsic factor(IF), R-protein (transcobalamin I), transcobalamin II, and transcobalamin III. IF is made by parietal cells, which lines the stomach mucosa. It plays a pivotal role in absorption of B12 from the gut (small intestine) into blood circulation [25.] R-protein, also known as haptocorrin or transcobalamin I is found in most body fluids . Though it has no known function, B12 bound to R-protein accounts for 80% of total plasma B12[26,27] and it may contribute to falsely high B12 measurements [28.]

Transcobalamin II, which is also known as holotranscobalamin (holoTC) when bound to B12, is found in blood plasma[25.] It is the only biologically active form of B12 and is

responsible for the transport of B12 to cell membrane receptors which are part of receptor mediated endocytosis) [25.] HoloTC accounts for 6-25% of total plasma B12 [22,29,30] and it is believed to be the most sensitive marker for depletion or repletion of B12 [26,30,31] Lastly, transcobalamin III is made by granulocytes [25,32] and has an unknown function [26.] However, it has clinical significance as elevated levels of it, as seen in chronic myelogenous leukemia, may cause falsely high measures of B12.[25]

2.2 **The biological roles of vitamin B12**

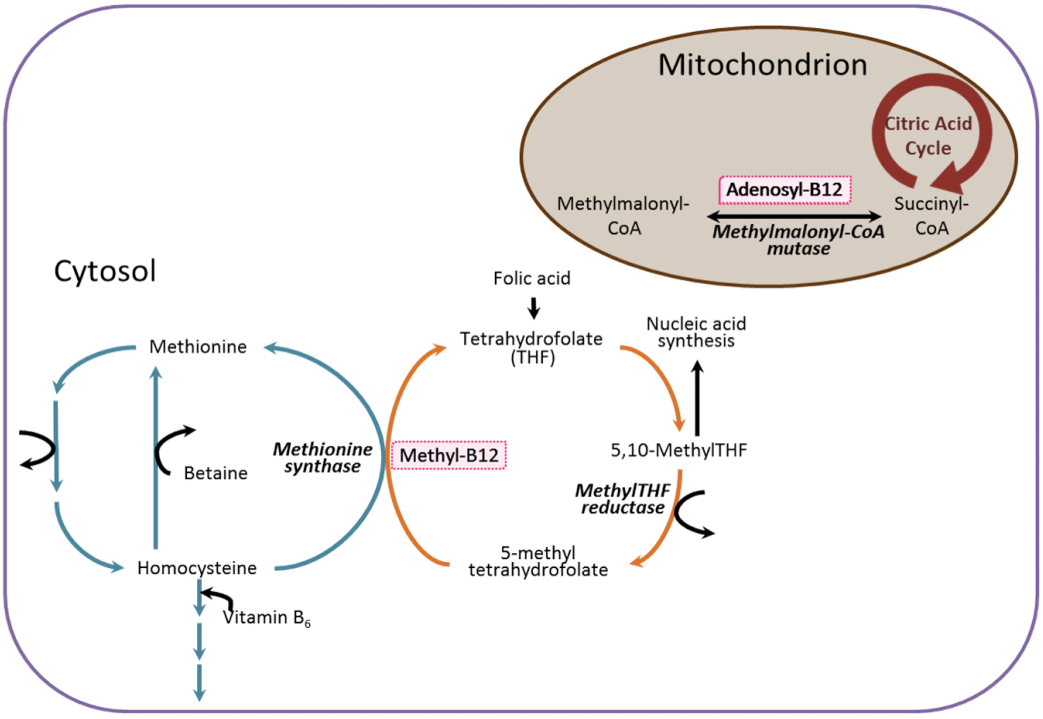


Fig 20: Simplified model of vitamin B12-dependent processes. [33]

In a simplified manner, the following section provides an overview of the biological processes in which B12 is involved which includes its relationship to energy metabolism, folate and vitamin B6 and is also summarized in Figure 19. Two forms of vitamin B12 are essential co-factors for biological processes:

1) Adenosyl-B12 in the mitochondria, which is for proper energy metabolism, and

2) Methyl-B12 in the cytosol of cells for methylation reactions which nucleic acid synthesis as well.

In the mitochondria, vitamin B12 is involved in oxidation reactions of both branch chain and odd chain fatty acids (e.g., threonine, valine, methionine) [34,35,36]Vitamin B12 (adenosylcobalamin) is required as a co-factor by the enzyme methylmalonyl-CoA mutase for conversion of methylmalonyl-CoA to succinyl-CoA which feeds into citric acid cycle for energy metabolism [34,35,36.] In vitamin B12 deficiency, methylmalonyl-CoA builds up and it gets converted to methylmalonic acid (MMA) [35,36] which is a myelin destabilizer. Although high MMA is a gold standard indication, it has drawbacks such as expense of measurement, the necessity for mass spectrometry, and the risk of concentration being raised by bacterial overgrowth, especially in impoverished nations[37].It also leads to less succinyl-CoA to feed into citric acid cycle yielding less efficient energy metabolism[35,36.]

In the cytosol, B12 is involved in the conversion of the amino acid, homocysteine(hcy) into the amino acid methionine. Vitamin B12 (cobalamin) is required as a co-factor by the enzyme methionine synthase for the transfer of a methyl group from 5-methyltetrahydrofolate (derived from folic acid) onto B12 (methylcobalamin) to form tetrahydrofolate. The methylcobalamin is then used as a methyl donor in the conversion of homocysteine to methionine, through methyl group addition. Generated methionine is required for the formation of s-adenosyl-methionine (SAM) which is a universal methyl donor for methylation reactions . Vit - B12 deficiency decreases the amount of methionine generated and downstream impacts every cell in the body from the formation of myelinneurotransmitters, phospholipidsto nucleic acid synthesis and RNA and DNA methylation [22,31,36]

Due to this metabolism, a by-product of vitamin B12 deficiency is elevated levels of homocysteine (Hcy), as it cannot be converted to methionine [38.] Raised levels of Hcy have been shown to be associated with dementia [39,40,] Alzheimer's [41,42,] carotid-artery stenosis[43,] stroke[44], a three-fold increased risk for myocardial infarction[43] and a four-fold increased odds ratio for venous thrombosis [45,46.] While the association doesn't infer causation, a recent meta-analysis provides supporting evidence for an association between low vitamin B12 & folic acid with high homocysteine levels[47.] As such, elevated homocysteine levels may be a modifiable risk factor amenable to vitamin B12 and/or folate therapy.

While vitamin B12 maintains homocysteine levels, so does vitamin B6 and another compound, betaine which has a similar action. Vitamin B6 is responsible for transamination of hcy to cysteine, thus improved B6 status can also reduce elevated Hcy levels or may elevate Hcy levels in the case of its deficiency. In the cytosol of most cells , betaine is present and is responsible for the conversion of hcy to methionine via an alternative pathway other than through vitamin B12. While betaine can help save from the effect of B12 deficiency by preventing elevations of hcy[48,49,] but it is not present in the brain, which may increase the importance for adequate vitamin B12 status with respect to healthy brain function.

Sources of vitamin b12 and recommended daily allowance (RDA)

Meat and dairy products are the only naturally occurring sources as it is created by microorganisms. The amount per serving varies depending on the source. For example a 250 mL serving of skim milk contains 1.3 mcg; beef liver, tuna, and chicken contain between 52.9-64.4 mcg, 8.2-9.3 mcg, and , 0.2-0.3 mcg of B12 per 2.5 oz (75 g) serving, respectively; 2 large eggs contain 1.5-1.6 mcg, Plant-based foods require fortification.[50]

Vegetarians are at the increased risk of developing vitamin B12 deficiency. Daily required adult dose is around 1-3 microgram, which is only about 0.1% of total body store. The total body stores are close to 2-3 milligram. So the total body store is sufficient for almost half a decade, in the absence of external supplies.[50]

AGE	MALE (mcg)	FEMALE (mcg)	PREGNANCY (mcg)	LACTATION (mcg)
0 – 6 months	0.4	0.4		
7–12 months	0.5	0.5		
1 – 3 years	0.9	0.9		
4 – 8 years	1.2	1.2		
9 – 13 years	1.8	1.8		
14 + years	2.4	2.4	2.6	2.8

Table 2 – RDA of Vitamin B12 as per different age groups

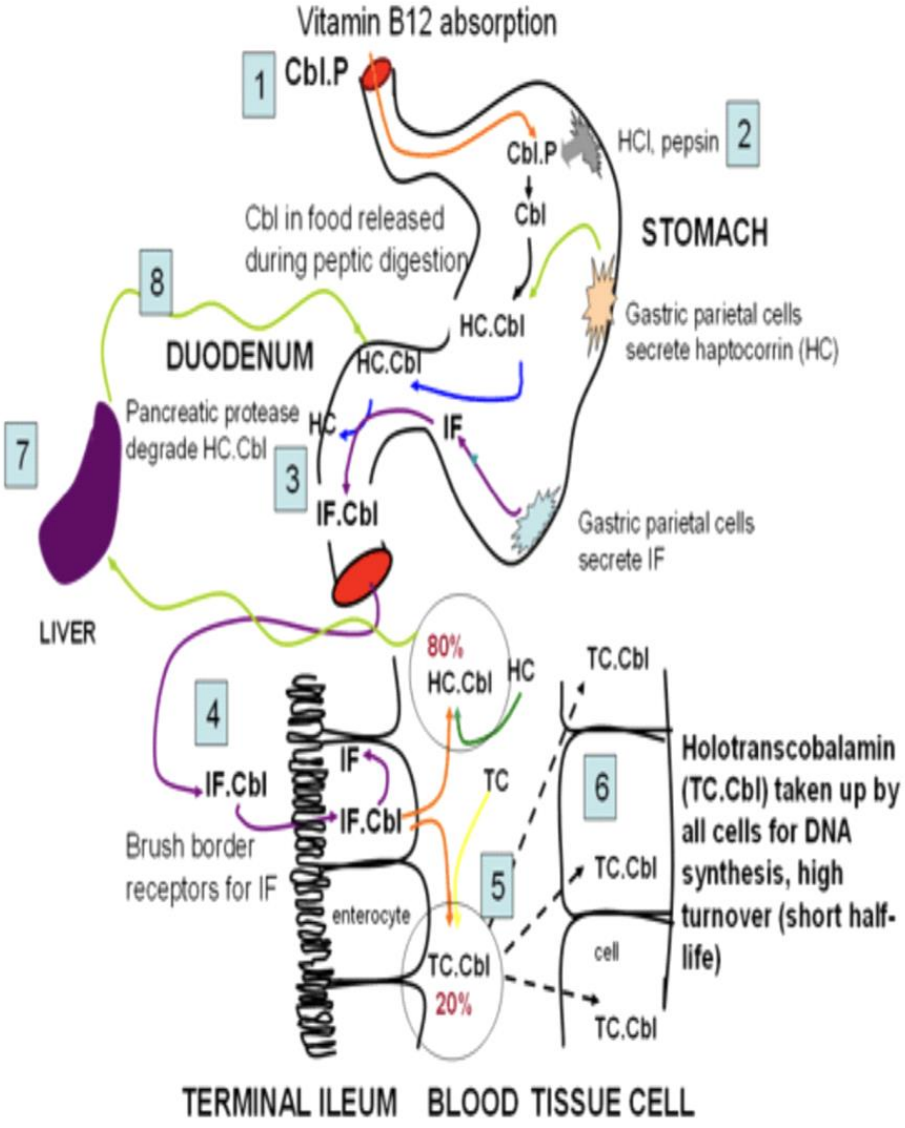
ABSORPTION AND TRANSPORT[50]

There are 2 mechanisms by absorption. Passive process occurs through small intestine mucosa. But it is highly inefficient and . Thus the normal physiological absorption process occurs by active diffusion at the terminal ileum in the presence of gastric IF.

Cobalamin in the diet is separated from its protein complexes by the action of gastric and small intestinal enzymes. Then it binds to the R- binder with which it is transported to the terminal ileum. IF is secreted from stomach parietal cells, than it combines with cobalamin and forms IF-Cobalamin complexes which are than transported to terminal ileum in the gut. This complex attaches itself to receptor cubilin which is an endocytic receptor protein which helps in the translocation of complex into the enterocytes, where the IF is destroyed. With a lag period of six hours cobalamin than appears in the portal circulation attached with transcobalamin II. High amount of cobalamin normally undergoes enterohepatic circulation (EHC) from the denuded intestinal epithelial cells.

Hence, the risk of this B12 deficiency is higher in patients with malabsorption as compared to vegetarians. The transport of cobalamin occurs in one to one molecule manner. Transcobalamin I is formed from the granules of neutrophils and it has no primary role in transport of cobalamin into the tissues.

The major protein responsible for peripheral transport is transcobalamin II which is produced from the liver endothelial cells, ileal enterocytes and macrophages. It takes up cobalamin to areas of high demand like marrow, placenta.



VITAMIN B12 ABSORPTION

Fig 21: Representation of Vitamin B12 absorption

Common causes for Deficiency

Generally, all factors fit into one of the following groups: Inadequate intake, dysfunction of food-cobalamin absorption or dysfunction of transport.

Inadequate intake of B12 is of concern in a strict vegan diet where natural sources of food B12 are avoided. Though generally adequate intake is not a concern in older individuals, those living in LTC(long term care) may additionally be at increased risk of inadequate intake of B12 due to the high prevalence of malnutrition ranging between 30-70% [23.] It could be hypothesized that since older adults living in LTC are more likely to have low food B12 intake and it is impossible for this population to normalize their B12 levels just by food alone, they are certainly at an increased risk for reduced B12 levels, especially when they do not take supplements.

A review study identified the leading cause of B12 deficiency in older adults to be food cobalamin malabsorption, accounting for 60-70% of cases [23.] Food-cobalamin malabsorption refers to the inability to adequately break B12 apart from food or from transport proteins[23,51] and can be caused by multiple factors such as: gastric atrophy either related or unrelated to H. pylori infections , bacterial overgrowth , alcoholism or use of certain medications like H2 blockers , PPIs or metformin [29,52,53.] Pernicious anemia occurs in those who lack IF; older adults with pernicious anemia accounted for 15-33% of B12 deficiency cases [23,29,33.] There are diseases that can impact absorption more broadly such as lymphoma, Crohn's disease, celiac disease, and may also cause B12 deficiency[23.]

Heredity of rare disease and genetic factors also play a role in B12 deficiency though to a much lesser extent in older adults as most appear to be autosomal recessive. Imerslund-Grasbeck syndrome, a rare disease often diagnosed in the initial years of life, impacts intestinal transport of B12 in which cell surface receptors which are responsible for receptor-mediated endocytosis fail to function properly[54.]

Indian scenario

As compared to a western diet, there is very minimal B12 in the average Indian diet, which is mostly restricted to vegetarian. There are various other factors, which could potentially cause malabsorption of B12. Many of them have malabsorption due to subclinical, clinical, or treated ileocecal tuberculosis, chronic pancreatitis or hepatobiliary dysfunction. Majority of our ailing population is now on PPI, metformin, or certain drugs, due to increasing morbidities and also the body stores of B12 are invariably poor. Even those who consume meat and do get B12 but do not get FA through diet because majority do not take fruits or raw vegetables in there routine diet. To top it all, almost all in India destroy FA by cooking, and we have a situation where noone gets B12 and FA in there diet by plan, and if someone gets any of them, it is only by chance . In addition, those who get B12 do not get FA on a regular basis. [55]

Symptoms of B12 deficiency:

If left untreated, it is likely to result in the classical pictures of hematologic or neurologic complications. 75 to 90% of those with B12 deficiency do have neurologic symptoms and around 25% of patients with neurologic symptoms might not present with any hematologic abnormalities [22,23.] Neurologic symptoms may result in paralysis from either subacute combined degeneration of the spine or funicular spinal cord disease(SCDS), unsteady gait

or skin numbness[22]. Hematologic abnormalities include pernicious anemia and macrocytic anemia.

If left untreated, the neurological damage becomes irreversible. However there is evidence which suggest there is a window period of opportunity in which to reverse these symptoms highlighting the importance of diligent testing (in the absence of symptoms) and treatment of deficient cases[56]. Additionally, symptoms relating to subclinical B12 deficiency are diverse and varied making it difficult to pinpoint B12 status as the potential cause. These symptoms include: lethargy or fatigue, depression, cognitive decline or dementia, increased confusion, forgetfulness, peripheral neuropathy, osteoporosis [35,57,58]. With such varied symptoms, many of which affecting the ability to live life normally, routine testing and treatment when appropriate is important.

Haematological effects

The rise in mean cell volume (MCV) occurs earlier than the development of anemia in cases of vitamin B12 or folate deficiency. The detection of Macrocytosis during the pre auto analyzer era was not very frequent. But in this period, it is very easy to detect the macrocytosis with the help of these automated analyzer. The clinical picture of vitamin B12 deficiency was not detected hematologically previously. Now it becomes easier to pick the B12 deficiency cases with the hematological picture as well. " An unexplained rise in MCV of 5fl or more even within the normal age should also attract suspicion". Dimorphic anemia is an entity which is the coexistence of both iron deficiency anemia as well as vitamin B12 deficiency. In the presence of these blood picture the classical vitamin B12 blood picture will not be seen.

“The earliest classical picture which is seen in the peripheral blood smear in the case of B12 and folate deficiency is Neutrophilic hypersegmentation. >5% of the neutrophils with 5 lobes or >1% of the neutrophils with 6 lobes is highly suggestive of vitamin B12 deficiency”. The smear picture can be similar in case of a myelodysplasia as well. In extreme deficiency status, the DNA synthesis is affected to such an extent that, pancytopenia can occur as well in such conditions. Pancytopenia is a relatively common presentation, though thrombocytopenia is not severe enough to cause clinical picture like bruising, bruising would occur only if another coexisting disorder of platelet dysfunction, vascular insult – due to vasculitis or Vitamin C deficiency or infections. The anemia and low leukocyte count predispose to infections due to impaired bactericidal function of phagocytes.

The Suspected Correlation:

In some subset of dengue patients recovery from thrombocytopenia is swift, while in other subset it may take several days.There may be other unknown factors that are contributing to thrombocytopenia and its slow recovery, during acute hematopoietic stress.
Hence, identifying and correcting contributory factor may probably cutdown duration of admission in patients with dengue fever.

MATERIALS AND METHODS

MATERIALS AND METHODS

SOURCE OF DATA:

- The study includes dengue positive patients admitted as inpatients in Department of Medicine of BLDE(deemed to be University)Shri B. M. Patil Medical College Hospital and Research Centre, Vijayapura.
- The patients were informed about study in all respects and informed consent was obtained.
- Period of study - from NOVEMBER 2019 TO AUG 2021.

Method of collection of Data (Including sampling procedures):

Patient admitted with complain of fever with clinical suspicion of dengue fever underwent dengue screening.All the diagnosed cases of dengue fever either screened positive in our hospital or diagnosed outside were selected for study.

Information was collected from each patient through a pre-tested proforma meeting the objectives of the study. Purpose of the study was carefully explained to patients and consent was taken.

Data collection by clinical history, examination and investigations was done. The details were recorded in the proforma. Serum vitamin B12 levelsand daily platelet count were measured.

For Vitamin B12 estimation 3 ml of fasting blood sample was collected in a plain vial and it was estimated using VITROS Vitamin B12 reagent packs and VITROS Vitamin B12 calibators on VITROS 3600 immunodiagnostic system and VITROS 5600 integrated system. This test is based on competitive binding immunoassay technique on principle of Chemiluminescence.

For diagnosis of dengue , Dengue Day 1 Test kit which is a rapid solid phase immune-chromatographic test was used for the qualitative detection of Dengue NS1 antigen and differential detection of IgM and IgG antibodies to dengue virus in human serum/plasma.[59]

As per the results of two different studies the NS1 strip rapid diagnostic tests has a specificity of 100% and 99% respectively.[59,60] The “Dengue Day 1 Test” which is used in this study has a sensitivity and specificity of 96% and 98% respectively for Dengue NS1 Ag and, 95% & 97% for Dengue IgM/IgG antibody.[59]

For complete blood count and daily platelet estimation 2 ml of blood was collected in EDTA vial.

For analysis purpose patients were divided into three groups according to B12 levels, viz, B12<150 pg/L, B12<151-300 pg/L and B12>300 pg/L.

Daily platelet count were measured from day of admission until recovery (1.5 lakhs/ml) / Discharge / Death.

SAMPLE COLLECTION

Oral and written consent were taken from the subjects prior to the collection of specimens.

INCLUSION CRITERIA:

1. Dengue positive for NS1 antigen/IgM/IgG / ELISA.

EXCLUSION CRITERIA:

Patients with -

1. Primary Hematological disorders

2. Septicemia secondary to causes other than dengue

3. Use of drugs which causes thrombocytopenia like heparin, chemotherapy, valproic acid, furosemide, penicillin, quinidine, quinine, sulphonamides.

4. Known underlying malignancy affecting blood cell counts.

SAMPLE SIZE:

With Anticipated correlation coefficient between B12 and no. of hospital stay 0.468[2,] at 99%confidence level and 90% power in the study, the sample size worked out is 61.

Formula used is

$$N=\left[\left(\frac{Z_\alpha+Z_\beta}{C}\right)\right]^2+3$$

$$C=0.5*\ln\left[\frac{1+r}{1-r}\right]$$

STATISTICAL ANALYSIS:

Patients characteristics will be presented using Mean±SD, percentages and Diagrams.

Different groups of Vitamin B12 in relation to platelet recovery in Dengue patients were compared with ANOVA TEST. Correlation between the variables were found by Correlation coefficient.

LIST OF INVESTIGATIONS

1 Dengue serology - NS1 antigen, IgM, IgG. (ELISA If needed in clinically suspicious cases with negative rapid detection test.)

2 CBC,

3 Peripheral smear,

4 Fasting vitamin B12 levels,

5 Daily platelet count until recovery(1.5 lakhs/ml) / Discharge, Death

6 Serum electrolytes,

7 URINE – Albumin, sugar, epithelial cells, pus cells, casts.

8 Other relevant investigations to rule out causes of thrombocytopenia other than dengue.

Along with the above investigations other relevant investigations were performed if required.

OBSERVATION AND RESULTS

OBSERVATION AND RESULTS :

A hospital based prospective study was conducted to evaluate the suspected correlation of thrombocytopenia recovery with serum vitamin B12 levels in dengue fever patients.

Initial evaluation had total of 65 dengue positive patients out of which 2 patients had mixed infection of dengue with malaria and 2 patients were transfused SDP/RDP because of bleeding manifestations. Hence, the final study included total of 61 dengue positive patients.

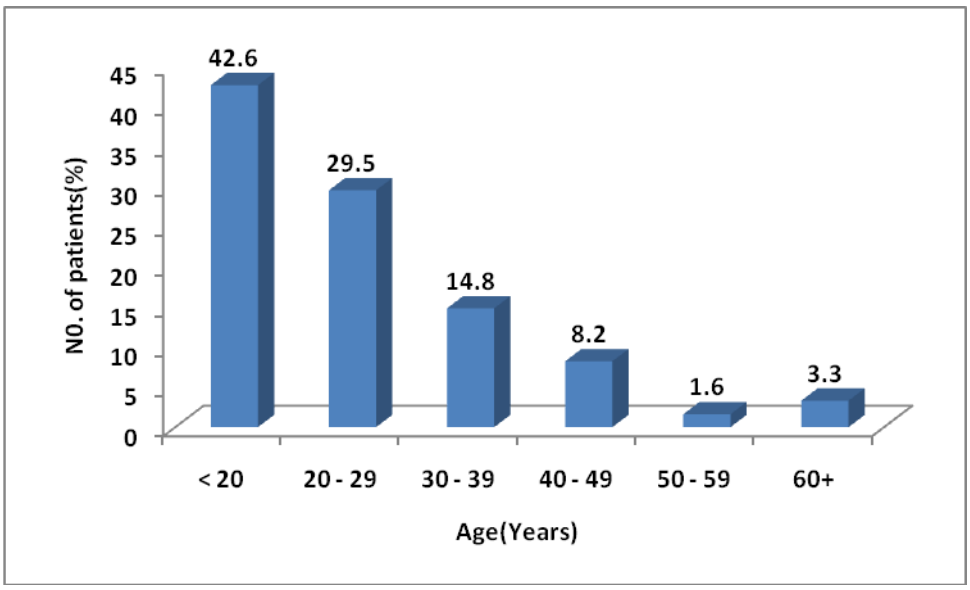
Distribution of patients according to age :

Table3:Distribution of patients according to Age(Years)

Age(Years)	No. of patients	Percentage
< 20	26	42.6
20 - 29	18	29.5
30 - 39	9	14.8
40 - 49	5	8.2
50 - 59	1	1.6
60+	2	3.3
Total	61	100.0

It was observed that majority of the patients belonged from the age groups of teenage to young adults probably because of there extra environmental exposure as compared to older age groups.

Out of all 42.6% of patients belong to age group of less than 20 years while 29.5 % of patients belongs to the age group from 20 to 29 years.

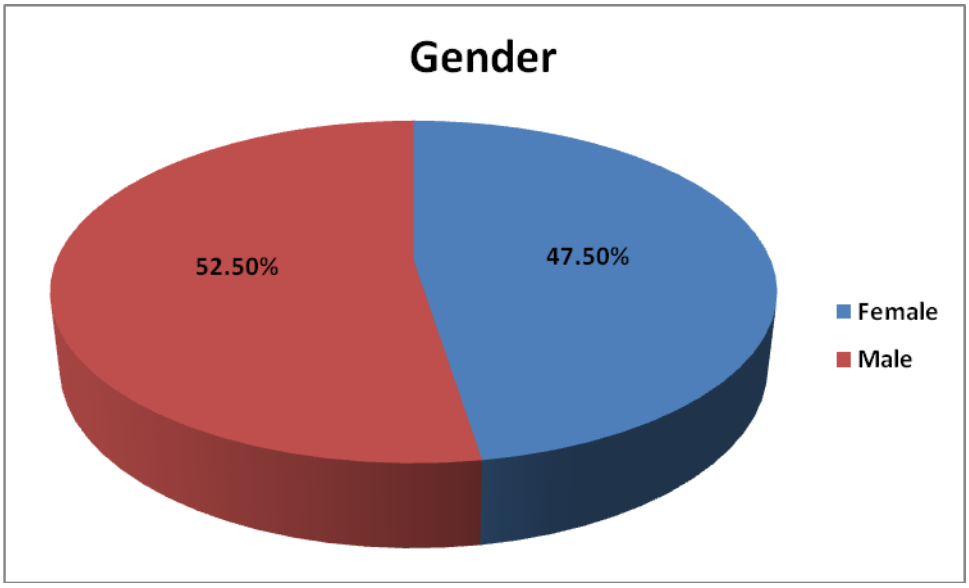


Graph 1 :Distribution of patients according to Age (Years)

Distribution of patients according to Gender:

Table 4: Distribution of patients according to Gender

Gender	No. of patients	percentage
Female	29	47.5
Male	32	52.5
Total	61	100.0



Graph 2: Distribution of patients according to Gender

It was observed that the gender inclination was not significant and 52.5% of patients in the study were male (32 in number) while 47.5% of patients were female (29 in number).

Vitamin B12 group

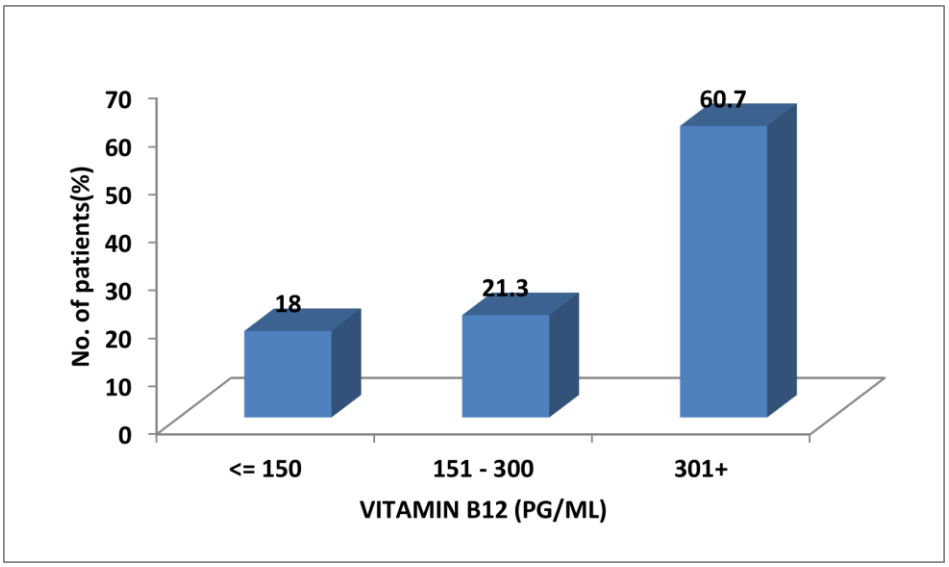
For an easier comparison patients were divided into three groups according to there serum vit B12 levels . with first group of patients having serum B12 levels less than 150 pg/ml i.e severely deficient and it was observed that 18% of total patients fell into this group.

Second group was of patients having serum B12 levels from 151 – 300 pg/ml i.e borderline normal and out of all, 21.3% of patients were in this category.

Third group was of patients having B12 levels of more than 300 pg/ml i.e adequate stores and 60.7% of patients were in this category.

Table 5:Distribution of patients according to levels of Vitamin B12 (pg/ml)

VITAMIN B12 (PG/ML)	No. of patients	Percentage
<= 150	11	18.0
151 - 300	13	21.3
301+	37	60.7
Total	61	100.0



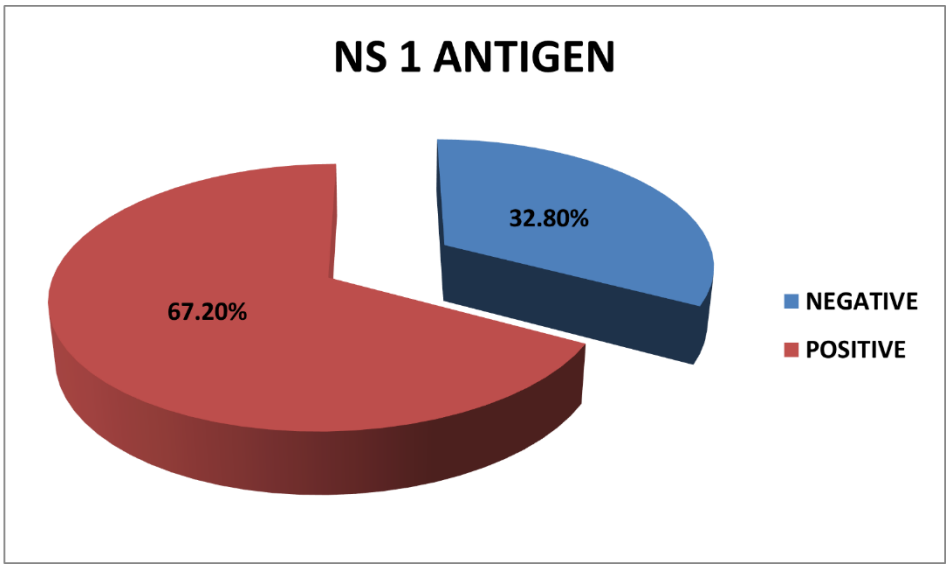
Graph 3 :Distribution of patients according to levels of Vitamin B12 (pg/ml)

DENGUE SEROLOGY

The patients were screened for Dengue fever with both primary NS1 antigen as well as antibody serology (IgM and IgG).

Table6:Distribution of patients as per NS 1 ANTIGEN positivity

NS 1 ANTIGEN	No. of patients	percentage
NEGATIVE	20	32.8
POSITIVE	41	67.2
Total	61	100.0



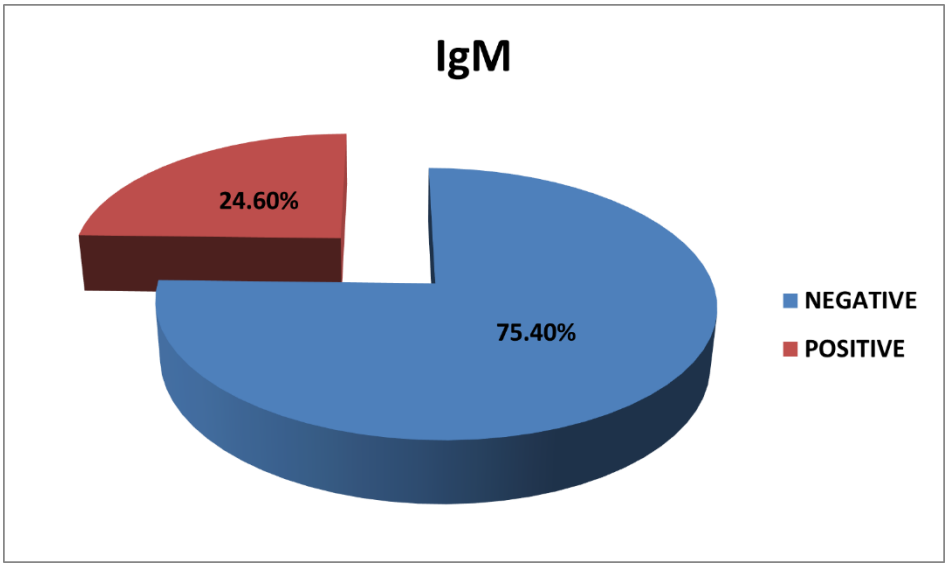
Graph 4 :Distribution of patients as per NS 1 ANTIGEN positivity

Out of all 67.2% of patients were found to be NS1 antigen positive that is 41 patients were in there primary infective stage of disease.

Table7:Distribution of patients as per IgM positivity

IgM	No. of patients	Percentage
NEGATIVE	46	75.4
POSITIVE	15	24.6
Total	61	100.0

A total of 24.6% of patients were found to be IgM positive irrespective of there NS1 antigen status.

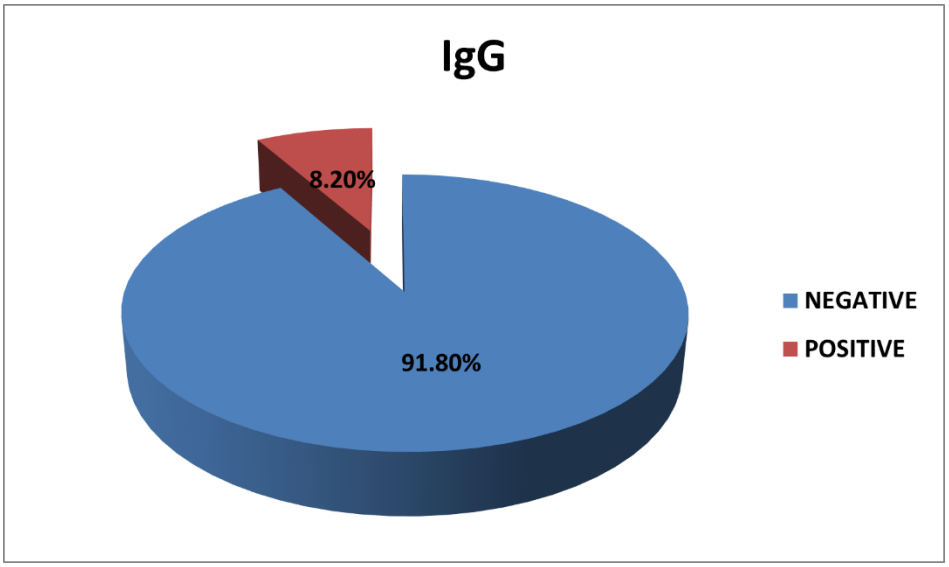


Graph 5 :Distribution of patients as per IgM positivity

Table8:Distribution of patients as per IgG positivity

IgG	No. of patients	percentage
NEGATIVE	56	91.8
POSITIVE	5	8.2
Total	61	100.0

Out of total only 8.2% of patients were found to be IgG positive irrespective of there antigen or IgM status , showing that only a small percentage of patients were in there recovery phase.



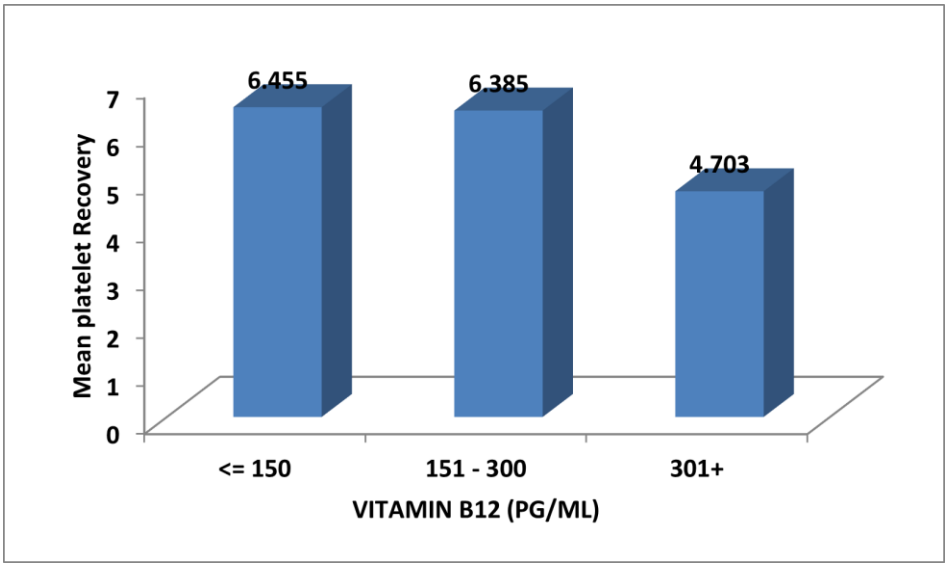
Graph 6 :Distribution of patients as per IgG positivity

The correlation with platelet recovery

Table9:Correlation of serum Vitamin B12 (pg/ml) vs Platelet recovery

VITAMIN B12 (PG/ML)	No. of patients	PLATELETRECOVERY		ANOVA test	P value
		Mean	Std. Deviation		
<= 150	11	6.455	2.621	4.934	0.011*
151 - 300	13	6.385	2.142		
301+	37	4.703	1.884		
Total	61	5.3770	2.215		
*:Statistically significant					

Considering the platelet recovery threshold as platelet count of >1.5 lakh/cumm or till the time of discharge , different groups of patients according to there Vitamin B12 levels were compared and correlation was established.



Graph 7 :Correlation of serum Vitamin B12 (pg/ml) vs Platelet recovery

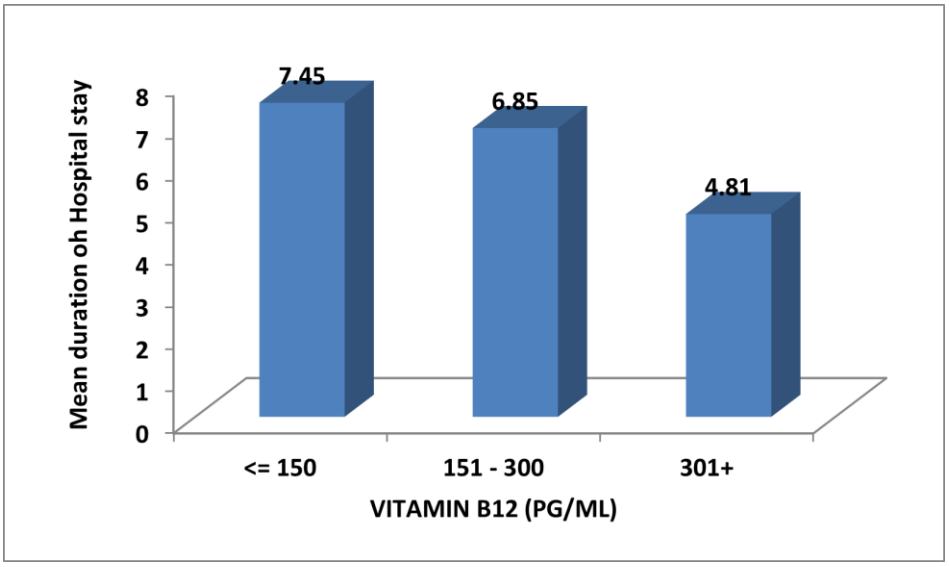
It was observed that group of patients with B12 levels less than 150 pg/ml took the maximum time to recover in terms of there platelet counts , and on the other end group of patients having adequate stores of B12 levels i.e more than 300 pg/ml recovered faster relatively , and the correlation established was found to be statistically significant.

Correlation with duration of Hospital stay

Table10 :Correlation of Vitamin B12 (pg/ml) vs no. of days of Hospital stay

VITAMIN B12 (PG/ML)	No. of patients	Duration of Hospital Stay		Kruskal-Wallis Test	P value
		Mean	Std. Deviation		
<= 150	11	7.45	2.505	14.666	0.001*
151 - 300	13	6.85	1.908		
301+	37	4.81	1.793		
Total	61	5.72	2.244		
*:Statistically significant					

As the platelet recovery took time it was expected to have a similar relation with hospital stay as well.



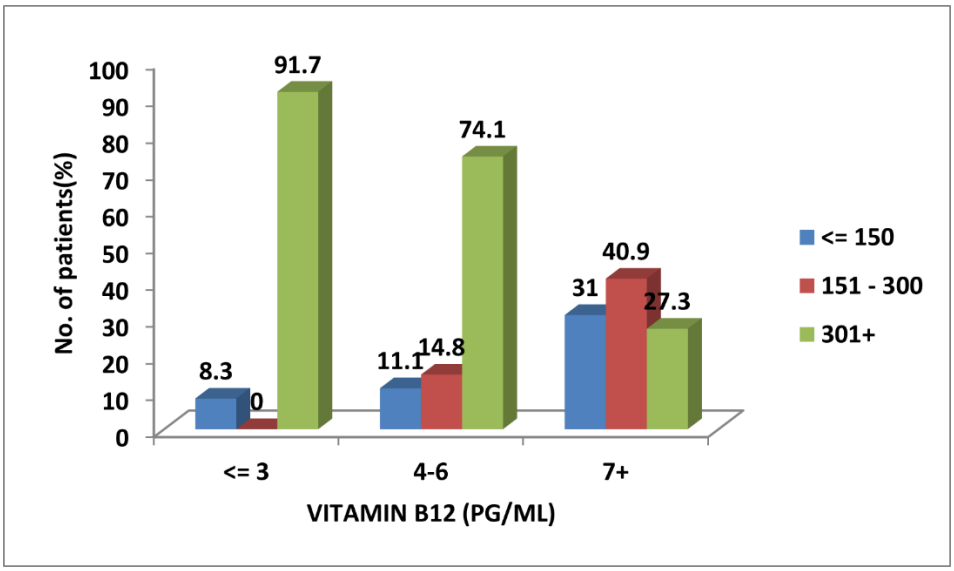
Graph 8 :Correlation of Vitamin B12 (pg/ml) vs no. of days of Hospital stay

The mean duration of hospital stay in Vit B12 deficient group was found to be 7.45 days while on the other end of spectrum, group with adequate stores of B12 had a mean stay of 4.81 days.

Table 11:Correlation of VitaminB12 (pg/ml) vs no. of days of Hospital stay in order

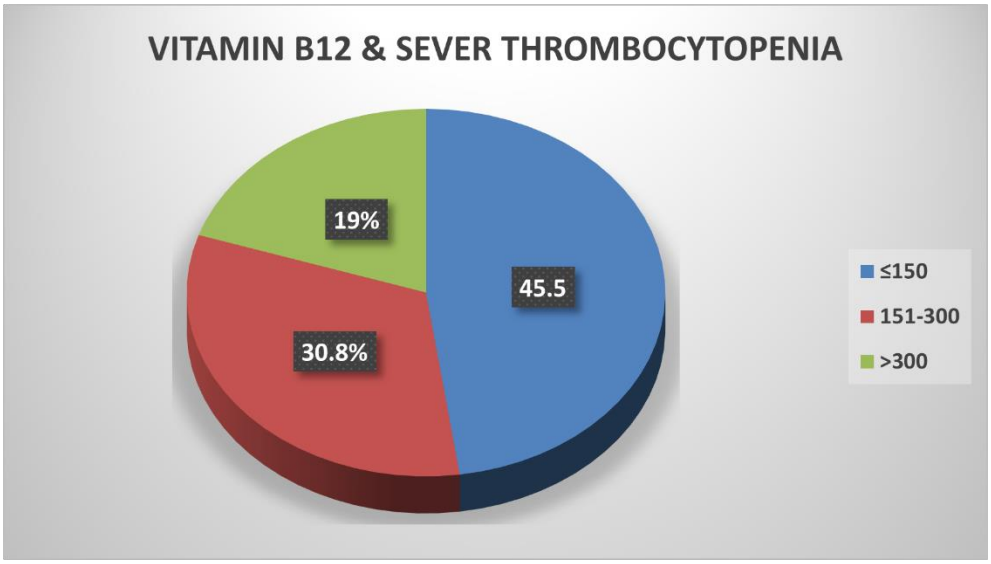
VITAMIN B12 (PG/ML)	NO. OF DAYS OF HOSPITAL STAY				Chi square test	P value
	<= 3	4 - 6	7+	Total		
<= 150	1	3	7	11	17.465	0.002*
	8.3%	11.1%	31.8%	18.0%		
151 - 300	0	4	9	13		
	0.0%	14.8%	40.9%	21.3%		
301+	11	20	6	37		
	91.7%	74.1%	27.3%	60.7%		
Total	12	27	22	61		
	100.0%	100.0%	100.0%	100.0%		
*:Statistically significant						

When the hospital stay was further categorized depending on the number of days it was observed that amongst the vit b12 deficient group 31.8% patients ended up staying for more than 7 days in hospital while on the other end of spectrum in group of patients with adequate vitamin b 12 levels , out of all 91.7% of the patients got discharged within 3 days od admission.



Graph 9 :Correlation of Vitamin B12 (pg/ml) vs no. of days of Hospital stay in order

Vitamin B12 and severe thrombocytopenia



Graph 10 : Occurrence of severe thrombocytopenia in different groups of Vitamin B12

As it was seen till now that recovery of platelet count was correlating with the serum levels of vitamin B12 but simultaneously one more observation was made that patient group with Vitamin B12 levels less than 150 pg/ml was more prone to go for severe thrombocytopenia i.e less than 20,000.

Table 12:Distribution of patients in different groups as per levels of serum Vitamin B12 (pg/ml)

VITAMIN B12 (PG/ML)& SEVER THROMBOCYTOPENIA	No. of patients	percentage
<= 150 (n=11)	5	45.5
151 – 300 (n=13)	4	30.8
301+(n=37)	7	19

It was noted that 45.5% patients of the vitamin B12 deficient group went for severe thrombocytopenia at one or the other stage of disease, in group B with B12 levels between 151

and 300 pg/ml 30.8% went for severe thrombocytopenia while in group C who had B12 levels of more than 300 pg/ml only 19% of them went for severe thrombocytopenia.

DISCUSSION

DISCUSSION

As we all are aware already that often the primary reason for admission and monitoring in dengue patients is thrombocytopenia and the fear associated with the possible complications of thrombocytopenia. And in majority of scenarios duration of admission and planning of discharge is all done around platelet count of the patient. And the so called safe level is considered as the increasing trend of daily platelet count along with counts reaching the lower limit of normal platelet count.

In several studies it has been observed that there is no direct correlation between severity of thrombocytopenia and dengue complications, but it has been noticed that in some subset of dengue patients the recovery from thrombocytopenia is relatively swift while in other subset it may take a prolonged period to recover.

Hence, this was the unexplained picture which framed the basis of this study and that is why we started this study with a presumption that there might be other factors that may be playing an unexplored significant role in thrombocytopenia and its slow recovery during the acute hematological stress created by the Dengue disease. And as we all know on one end there lies the risk of complications of thrombocytopenia while on the other hand prolonged hospital stay creates an extra load on the infrastructure of health system with more expenses.

So the bigger goal behind the study being identifying those contributory factor whose correction might help us cut down on duration of admission in patients with dengue fever.

Serum Vitamin B12 levels are not usually tested in patients being treated for thrombocytopenia secondary to spectrum of dengue viral infection.Vitamin B12 deficiency is known to cause

anemia and leukopenia but with severe deficiency it is notorious enough to cause thrombocytopenia as well and as the Indian population comes as one of the vulnerable population suffering from vit B12 deficiency the scenario becomes even more relevant for the study. With this background we started the study to test the hypothesis that vitamin B12 level might have a correlation with severe thrombocytopenia and its recovery.

In our study the most common age group was of patients less than 20 years old which formed the 42.6% composition out of all followed by 29.5% of patients coming from age group 20 to 29 years of age. While 47.5% of patients were female , 52.5% of patients were male. While in the study conducted by **Kevin R. Porter et al**[61]the most affected age group was 26 – 35 with 40% of the total patients belonged to that age group and only 5 % of patients belonged to the age group of 15-25 years.

Although the prevalence of Vitamin B12 deficiency is presumed higher in Indian population because of strict vegetarian eating lifestyle , in our study 18% of the patients were found to be significantly deficient with levels less than 150 pg/ml while 21.3% of patients with borderline low reserve status having B12 levels between 151 to 300 pg/ml. While in the study conducted by **Singla R. et al**[62]the percentage of population having Vitamin B12 levels of less than 200 pg/ml is 31.4 % and only 26% of population was observed to sufficient in B12 levels that is more than 300 pg/ml. And in the study done by **Chakraborty et al**[63]the prevalence of vitamin B12 deficiency in the total study population was 32.4% (threshold levels < 150 pg/ml)

As the dengue serology was run for all the patients including NS1 antigen , IgM and IgG. 75.5 % of patients showed positivity for NS1 antigen , 24.60% of patients showed positivity for IgM antibody and 8.2% of patients showed positivity for IgG antibody.

On this particular correlation there was only one study which was conducted till the time this research was done hence comparisons were done particularly with that study.

In our study when vitamin B12 levels were compared with platelet recovery it was observed that the group of patients who is deficient in B12 took a mean 6.45 days to show platelet recovery while the group with B12 levels between 150 to 300 pg/ml took mean 6.38 days to show platelet recovery and the group with vitamin B12 levels of more than 300 pg/ml took mean of 5.37 days to show platelet recovery and with ANOVA test P value was found out to be 0.011 making the correlation statistically significant.In study conducted by **SandeepTak et al[2]** the mean time for platelet recovery was 5.75 days in the Vitamin B12 deficient group while in the B123 adequate group the mean time for platelet recovery was found out to be 3.00 days reflecting a significant correlation in there study as well.

When the correlation of vitamin B12 and duration of hospital stay was being explored, it was expected to follow the pattern of platelet recovery as usually more the time of platelet recovery, more the duration of hospital stayits going to be. The deficient group with B12 levels less than 150 pg/ml showed a mean duration of hospital stay of 7.45 days, the second group with B12 levels between 150 and 300pg/ml showed a mean stay of 6.85 days while the group with B12 levels of more than 300 had the mean duration of stay of 4.81 days and using KRUSKAL-WALLIS test the P value was found out to be 0.001 making the correlation statistically significant.In study conducted by **SandeepTak et al[2]** the mean duration of hospital stay in the Vitamin B12 deficient group was 5.25 days while in B12 adequate group the mean duration of hospital stay was found to be 3.64 days and the correlation in this study as well was found to be statistically significant.

It was found that not only the recovery of platelet count is slow in deficient patient group but also the deficient age group are more prone for severe thrombocytopenia (platelet count less than 20,000/cumm.) and it was noted that 45.5% patients of group A went for severe thrombocytopenia while 30.8% patients of group B went for severe thrombocytopenia and only 19% patients of group C went for a platelet count of less than 20,000/cumm at some stage of the disease.

LIMITATIONS OF STUDY

As the size of study group was just 61 patients due to situation of pandemic during the phase of study, a larger size of the study group will surely reflect the bigger picture in a better and more clear way.

As the patients were included in the study irrespective of the phase of illness, we might have missed the stage of lowest platelet count in some patients as some of them might be already in the recovery phase of illness.

A second half of this study could have been a set of patients or another arm of the study in which patients receives injectable Vitamin B12 and a response can be recorded to see whether supplementation affects the thrombocytopenia recovery in any way or not. This lay out can be a follow up study of this project which can further help in strengthening the suspected correlation of Vitamin B12 levels with thrombocytopenia recovery in Dengue fever patients.

CONCLUSIONS

CONCLUSIONS

Vitamin B12 deficiency is, may be a major contributing factor for the development of severe thrombocytopenia in dengue fever patients. During the acute hematopoietic stress created in dengue illness the bone marrow of vitamin B12 deficient subset of patients lags behind to compensate for falling platelets.

Severe Vitamin B12 deficiency delays the recovery of thrombocytopenia in Dengue fever patients.

Vitamin B12 deficiency also prolongs the duration of hospital stay in Dengue fever patients in comparison to those who have adequate Vitamin B12 levels creating an extra burden on patient as well as health infrastructure.

Vitamin B12 deficiency is still significantly prevalent in Indian population contributing to a spectrum full of problems. According to the data of this study 18% of the participants were severely deficient for Vitamin B12 while 21.3% of participants were low normal to borderline.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. National vector borne disease control programme(2014):Natonal guidelines for clinical management of fever.

2. Tak S, Geethu, Rathore JS, Charan SS, Bijarniya R, Lakhotia M. Severe thrombocytopenia in dengue fever and vitamin B12 level. J Assoc Physicians India. 2018;66(September):62–5.

3. Sivaprasad M, Shalini T, Balakrishna N, Sudarshan M, Lopamudra P, Suryanarayana P, et al. Status of Vitamin B12 and Folate among the Urban Adult Population in South India. Ann NutrMetab. 2016;68(2):94–102.

4. Walter K, Vaughn J, Martin D. Therapeutic dilemma in the management of a patient with the clinical picture of TTP and severe B 12 deficiency. BMC Hematol [Internet]. 2015;15(1):1–5. Available from: http://dx.doi.org/10.1186/s12878-015-0036-2

5. Panchabhai TS, Patil PD, Riley EC, Mitchell CK. When the picture is fragmented: Vitamin B12deficiency masquerading as thrombotic thrombocytopenic purpura. Int J Crit IllnInj Sci. 2016;6(2):89–92.

6. Kandel S, Budhathoki N, Pandey S, et al. Pseudo-thrombotic thrombocytopenic purpura presenting as multi-organ dysfunction syndrome: A rare complication of pernicious anemia. *SAGE Open Med Case Rep*. 2017;5:2050313X17713149. Published 2017 Jun 6. doi:10.1177/2050313X17713149.

7. Gubler DJ. Dengue/dengue haemorrhagic fever: history and current status. *Novartis Found Symp*. 2006;277:3-253. doi:10.1002/0470058005.ch2

8. Warkentien, Tyler &Pavlicek, Rebecca. (2016). Dengue Fever: Historical Perspective and the Global Response. Journal of Infectious Disease and Epidemiology. 2. 10.23937/2474-3658/1510015.

9. Chaturvedi UC, Nagar R. Dengue and dengue haemorrhagic fever: Indian perspective. *J Biosci*. 2008;33(4):429-441. doi:10.1007/s12038-008-0062-3.

10. World Health Organization and Tropical Diseases Research. Dengue: Guidelines for diagnosis, treatment, prevention and control. Geneva: World Health Organization; 2009: new edition.

11. Comprehensive guidelines for prevention and control of dengue and dengue haemorrhagic fever. Revised and expanded edition. (SEARO Technical Publication Series No. 60 ISBN 978-92-9022-387-0

12. Dengue and severe dengue [Internet]. Who.int. 2021 [cited 19 November 2021]. Available from: https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue

13. India M. DENGUE/DHF SITUATION IN INDIA :: National Vector Borne Disease Control Programme (NVBDCP) [Internet]. Nvbdcp.gov.in. 2021 [cited 19 November 2021]. Available from: https://nvbdcp.gov.in/index4.php?lang=1&level=0&linkid=431&lid=3715

14. Mutheneni, Srinivasa Rao & Morse, Andrew &Caminade, Cyril &Upadhyayula, Suryanaryana. (2017). Dengue burden in India: Recent trends and importance of climatic parameters. Emerging Microbes and Infections. 6. 10.1038/emi.2017.57.

15. Dash AP, Bhatia R, Kalra NL. Dengue in South East Asia: An appraisal of case management and vector control. Dengue Bulletin. 2012;36:1–12.

16. Kalayanarooj S. Clinical manifestations and management of dengue/DHF/DSS. Tropical Medicine and Health. 2011; 39 (4 suppl) 83–9

17. Simmons, C. P., Farrar, J. J., van Vinh Chau, N., & Wills, B. (2012). Dengue. *New England Journal of Medicine*, *366*(15), 1423–1432. https://doi.org/10.1056/nejmra1110265

18. Peeling RW, Artsob H, Pelegrino JL, et al. Evaluation of diagnostic tests: dengue. *Nat Rev Microbiol*. 2010;8(12 Suppl):S30-S38. doi:10.1038/nrmicro2459

19. Biswas, Ashutosh &Pangtey, Ghan& Devgan, V. & Singla, P. & Murthy, P. &Dhariwal, Akshay& Sen, P.K. & Baruah, Kalpana. (2015). Indian national guidelines for clinical management of dengue fever. 113. 196-206.

20. WHO. Dengue and severe dengue [Internet]. Who.int. World Health Organization: WHO; 2020. Available from: https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue

21. Deng SQ, Yang X, Wei Y, Chen JT, Wang XJ, Peng HJ. A Review on Dengue Vaccine Development. *Vaccines (Basel)*. 2020;8(1):63. Published 2020 Feb 2. doi:10.3390/vaccines8010063.

22. Grober, U., K. Kisters, and J. Schmidt, Neuroenhancement with vitamin B12-underestimated neurological significance. Nutrients, 2013. 5(12): p. 5031-45

23. Dali-Youcef, N. and E. Andres, An update on cobalamin deficiency in adults. Qjm, 2009. 102(1): p. 17-28

24.Oberley, M.J. and D.T. Yang, Laboratory testing for cobalamin deficiency in megaloblastic anemia. Am J Hematol, 2013. 88(6): p. 522-6.

25. Klee, G.G., Cobalamin and folate evaluation: measurement of methylmalonic acid and homocysteine vs vitamin B(12) and folate. Clin Chem, 2000. 46(8 Pt 2): p. 1277-83.

26. Herrmann, W. and R. Obeid, Utility and limitations of biochemical markers of vitamin B12 deficiency. European journal of clinical investigation, 2013. 43(3): p. 231-237.

27. Gimsing, P. and E. Nexo, Cobalamin-binding capacity of haptocorrin and transcobalamin: age-correlated reference intervals and values from patients. Clin Chem, 1989. 35(7): p. 1447-51.

28. Bell, I.R., et al., Relationship of normal serum vitamin B12 and folate levels to cognitive test performance in subtypes of geriatric major depression. J Geriatr Psychiatry Neurol, 1990. 3(2): p. 98-105.

29. Herrmann, W. and R. Obeid, Causes and early diagnosis of vitamin B12 deficiency. DtschArztebl Int, 2008. 105(40): p. 680-5.

30. Nexo, E. and E. Hoffmann-Lucke, Holotranscobalamin, a marker of vitamin B-12 status: analytical aspects and clinical utility. Am J Clin Nutr, 2011. 94(1): p. 359s-365s.

31. Herbert, V., Staging vitamin B-12 (cobalamin) status in vegetarians. Am J Clin Nutr, 1994. 59(5 Suppl): p. 1213s-1222s.

32. Baik, H.W. and R.M. Russell, Vitamin B12 deficiency in the elderly. Annu Rev Nutr, 1999. 19: p. 357-77.

33. Andres, E., et al., Vitamin B12 (cobalamin) deficiency in elderly patients. CMAJ, 2004. 171(3): p. 251-9.

34. Watkins, D. and D.S. Rosenblatt, Inborn errors of cobalamin absorption and metabolism. Am J Med Genet C Semin Med Genet, 2011. 157c(1): p. 33-44.

35. Solomon, L.R., Disorders of cobalamin (Vitamin B12) metabolism: Emerging concepts in pathophysiology, diagnosis and treatment. Blood Reviews, 2007. 21(3): p. 113-130.

36. Wolters, M., A. Strohle, and A. Hahn, Cobalamin: a critical vitamin in the elderly. Prev Med, 2004. 39(6): p. 1256-66.

37. Allen, L.H., How common is vitamin B-12 deficiency? The American journal of clinical nutrition, 2009. 89(2): p. 693S-696S.

38. Wong, C.W., Vitamin B12 deficiency in the elderly: is it worth screening? Hong Kong Med J, 2015. 21(2): p. 155-64.

39. Seshadri, S., et al., Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. N Engl J Med, 2002. 346(7): p. 476-83

40. Ravaglia, G., et al., Homocysteine and folate as risk factors for dementia and Alzheimer disease. Am J Clin Nutr, 2005. 82(3): p. 636-43.

41. Bottiglieri, T., K. Hyland, and E.H. Reynolds, The clinical potential of ademetionine (Sadenosylmethionine) in neurological disorders. Drugs, 1994. 48(2): p. 137-152.

42. Stanger, O., et al., Homocysteine, folate and vitamin B12 in neuropsychiatric diseases: review and treatment recommendations. Expert Rev Neurother, 2009. 9(9): p. 1393-412.

43. Selhub, J., et al., Association between plasma homocysteine concentrations and extracranial carotid-artery stenosis. N Engl J Med, 1995. 332(5): p. 286-91.

44. Giles, W.H., et al., Total homocyst(e)ine concentration and the likelihood of nonfatal stroke: results from the Third National Health and Nutrition Examination Survey, 1988-1994. Stroke, 1998. 29(12): p. 2473-7.

45. den Heijer, M., et al., Hyperhomocysteinemia as a risk factor for deep-vein thrombosis. N Engl J Med, 1996. 334(12): p. 759-62.

46. Ridker, P.M., et al., Interrelation of hyperhomocyst(e)inemia, factor V Leiden, and risk of future venous thromboembolism. Circulation, 1997. 95(7): p. 1777-82.

47. Shen, L. and H.F. Ji, Associations between Homocysteine, Folic Acid, Vitamin B12 and Alzheimer's Disease: Insights from Meta-Analyses. J Alzheimers Dis, 2015.

48. Bottiglieri, T., Homocysteine and folate metabolism in depression. Progress in NeuroPsychopharmacology and Biological Psychiatry, 2005. 29(7): p. 1103-1112.

49.Obeid, R., A. McCaddon, and W. Herrmann, The role of hyperhomocysteinemia and Bvitamin deficiency in neurological and psychiatric diseases. Clinical Chemical Laboratory Medicine, 2007. 45(12): p. 1590-1606.

50. Office of Dietary Supplements - Vitamin B12 [Internet]. Ods.od.nih.gov. 2021 [cited 19 November 2021]. Available from: https://ods.od.nih.gov/factsheets/VitaminB12-HealthProfessional/

51. Carmel R. 10 Malabsorption of food cobalamin. Baillière's Clinical Haematology [Internet]. 1995 Sep 1 [cited 2021 Nov 19];8(3):639–55. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0950353605802240

52. Dharmarajan TS, Kanagala MR, Murakonda P, Lebelt AS, Norkus EP. Do acid-lowering agents affect vitamin B12 status in older adults?. *J Am Med Dir Assoc*. 2008;9(3):162-167. doi:10.1016/j.jamda.2007.10.004

53. de Jager J, Kooy A, Lehert P, et al. Long term treatment with metformin in patients with type 2 diabetes and risk of vitamin B-12 deficiency: randomised placebo controlled trial. *BMJ*. 2010;340:c2181. Published 2010 May 20. doi:10.1136/bmj.c2181

54. Carmel R, Green R, Rosenblatt DS, Watkins D. Update on cobalamin, folate, and homocysteine. *Hematology Am Soc Hematol Educ Program*. 2003;62-81. doi:10.1182/asheducation-2003.1.62

55. Sasidharan P K. B12 deficiency in India. Arch Med Health Sci 2017;5:261-8

56. Ho, C., G.P. Kauwell, and L.B. Bailey, Practitioners' guide to meeting the vitamin B-12 recommended dietary allowance for people aged 51 years and older. J Am Diet Assoc, 1999. 99(6): p. 725-7.

57. Rosenberg, I.H. and J.W. Miller, Nutritional factors in physical and cognitive functions of elderly people. Am J Clin Nutr, 1992. 55(6 Suppl): p. 1237S-1243S.

58. Vogiatzoglou, A., et al., Cognitive function in an elderly population: interaction between vitamin B12 status, depression, and apolipoprotein E epsilon4: the Hordaland Homocysteine Study. Psychosom Med, 2013. 75(1): p. 20-9.

59.jmitra. Rapid Dengue Test Kit - Highly Sensitive | J Mitra & Co [Internet]. jmitra.co.in. [cited 2021 Nov 19]. Available from: http://jmitra.co.in/product-details/dengue-day-1-rapid-test-kit/

60. Mat Jusoh TNA, Shueb RH. Performance Evaluation of Commercial Dengue Diagnostic Tests for Early Detection of Dengue in Clinical Samples. *J Trop Med*. 2017;2017:4687182. doi:10.1155/2017/4687182.

61. Porter, Kevin & Beckett, Charmagne&Kosasih, Herman & Tan, Ratna&Alisjahbana, Bachti&Fianza, Pandji&Widjaja, Susana &Listiyaningsih, Erlin &Ma'Roef, Chairin& McArdle, James &Parwati, Ida &Sudjana, Primal & Jusuf, Hadi&Yuwono, Djoko &Wuryadi, Suharyono. (2005). Epidemiology of dengue and dengue hemorrhagic fever in a cohort of adults living in Bandung, West Java, Indonesia. The American journal of tropical medicine and hygiene. 72. 60-6. 10.4269/ajtmh.2005.72.60.

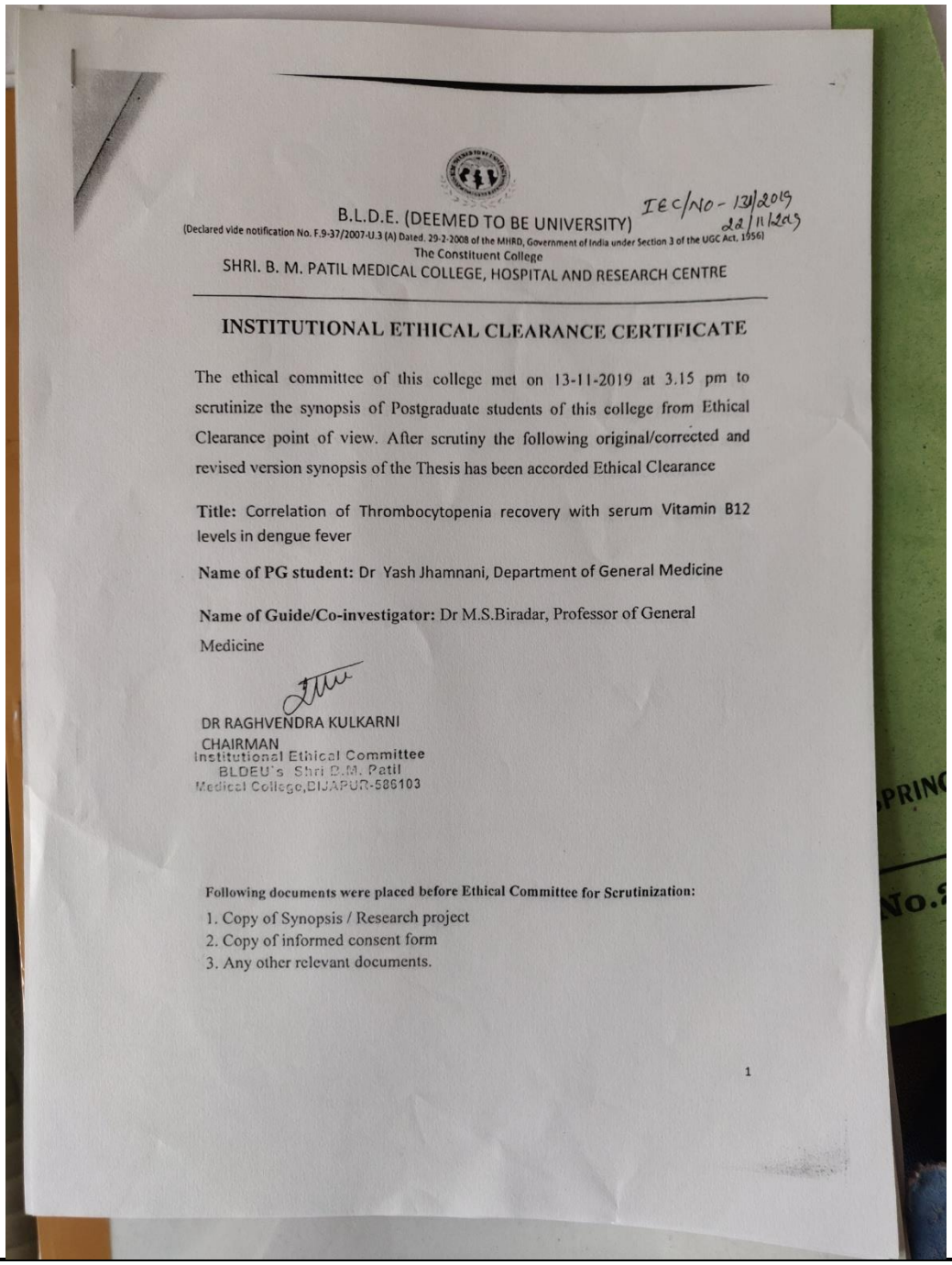
62. Singla R, Garg A, Surana V, Aggarwal S, Gupta G, Singla S. Vitamin B12 Deficiency is Endemic in Indian Population: A Perspective from North India. *Indian J Endocrinol Metab*. 2019;23(2):211-214. doi:10.4103/ijem.IJEM_122_19

63. Chakraborty S, Chopra M, Mani K, et al. Prevalence of vitamin B_{12} deficiency in healthy Indian school-going adolescents from rural and urban localities and its relationship with various anthropometric indices: a cross-sectional study. *J Hum Nutr Diet*. 2018;31(4):513-522. doi:10.1111/jhn.12541

ANNEXURES

ANNEXURE – I

ETHICAL COMMITTEE APPROVAL LETTER



B.L.D.E. (DEEMED TO BE UNIVERSITY) IEC/NO-13/2019 22/11/2019

(Declared vide notification No. F.9-37/2007-U.3 (A) Dated. 29-2-2008 of the MHRD, Government of India under Section 3 of the UGC Act. 1956)

The Constituent College

SHRI. B. M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The ethical committee of this college met on 13-11-2019 at 3.15 pm to scrutinize the synopsis of Postgraduate students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected and revised version synopsis of the Thesis has been accorded Ethical Clearance

Title: Correlation of Thrombocytopenia recovery with serum Vitamin B12 levels in dengue fever

Name of PG student: Dr Yash Jhamnani, Department of General Medicine

Name of Guide/Co-investigator: Dr M.S.Biradar, Professor of General Medicine

DR RAGHVENDRA KULKARNI
CHAIRMAN
Institutional Ethical Committee
BLDEU's Shri B.M. Patil
Medical College,BIJAPUR-586103

Following documents were placed before Ethical Committee for Scrutinization:

1. Copy of Synopsis / Research project
2. Copy of informed consent form
3. Any other relevant documents.

1

ANNEXURE – II

INFORMED CONSENT FORM

TITLE OF RESEARCH **:** CORRELATION OF THROMBOCYTOPENIA RECOVERY WITH SERUM VITAMIN B12 LEVELS IN DENGUE FEVER

GUIDE **: DR. BADIGER SHARANABASAWAPPA**

P.G.STUDENT **: DR YASH JHAMNANI**

PURPOSE OF RESEARCH:

I have been informed that the purpose of this study is to assess the correlation of thrombocytopenia recovery with serum vitamin B12 levels in dengue fever.

PROCEDURE:

I understand that I will undergo detailed history and clinical examination and investigations.

RISKS AND DISCOMFORTS:

I understand that there is no risk involved in this study and I may experience some pain during the above mentioned procedures.

BENEFITS:

I understand that my participation in this study will help to study thecorrelation of thrombocytopenia recovery with serum vitamin B 12 levels in dengue fever.

CONFIDENTIALITY:

I understand that the medical information produced by the study will become a part of hospital record and will be subjected to confidentiality and privacy regulation of hospital. If the data is used for publication the identity will not be revealed.

REQUEST FOR MORE INFORMATION:

I understand that I may ask for more information about the study at any time.

REFUSAL OR WITHDRAWAL OF PARTICIPATION:

I understand that my participation is voluntary and I may refuse to participate or withdraw from study at any time.

INJURY STATEMENT:

I understand in the unlikely event of injury to me during the study I will get medical treatment but no further medical compensation.

_____

(Signature of patient)

STUDY SUBJECT CONSENT FORM

I confirm that Dr. Yash Jhamnani has explained to me the purpose of this research, the study procedure that I will undergo and the possible discomforts and benefits that I may experience, in my own language.

I have been explained all above in detail in my own language and I understand the same. I agree to give my consent to participate as a subject in this research project.

SIGNATURE OF PARTICIPANT DATE

ANNEXURE – III: SCHEME OF CASE TAKING PROFORMA

BLDE (Deemed to be University)

SHRI B.M. PATIL MEDICAL COLLEGE

HOSPITAL AND RESEARCH CENTRE,

VIJAYAPURA

"CORRELATION OFTHROMBOCYTOPENIA RECOVERY

WITH SERUM VITAMIN B12 LEVELS IN DENGUE FEVER"

Name of the patient :

Age in years :

Sex :

Address:

Religion:

Occupation:

IP no/OP no:

Presenting Complaints :

Past history:

Personal history:

1 . Diet- Veg/Mixed

2. Sleep

3. Appetite

4. Bowel and Bladder Habits

5. Habits

Family history:

GENERAL PHYSICAL EXAMINATION :

Built :

Nourishment :

Ht(Cm) :

Wt(Kg) : BMI:

GENERAL EXAMINATION (HEAD TO TOE) :

Description of rashes if any -

Vital parameters a. Pulse :

b. BP :

c. Respiratory Rate :

d. Temperature

SYSTEMIC EXAMINATION:

ABDOMEN EXAMINATION

CARDIOVASCULAR SYSTEM

RESPIRATORY SYSTEM

CENTRAL NERVOUS SYSTEM

PATHOLOGY	
1) Dengue Serology	
2) Vitamin B12	
3) Serum electrolytes	
4) Urine examination	
5)Complete blood count:	
Hb	gm/dl
Total count	Cells/cumm
Differential count Neutrophils	%
Lymphocytes	%
Eosinophils	%
Basophils	%
Monocytes	%
ESR	

Platelet Count (daily)	Lakhs/cumm
6) Peripheral smear	

CONCLUSION :

Diagnosis -

Stage of disease -

Vitamin B12 level -

Lowest platelet count during disease course -

No. of days of hospital stay -

SIGNATURE

DATE:

	S.N	NAME	AGE(YEARS)	SEX	DIET(VEG/MIXED)	BODY MASS INDEX(KG/M2)	VITALS-	PULSE RATE(BPM)	SYSTOLIC BLOOD PRESSURE(MMHG)	DIASTOLIC BLOOD PRESSURE(MMHG)	TEMPERATURE(FAHRENHEIT)	RESPIRATORY RATE(CPM)	DENGUE SEROLOGY -	NS 1 ANTI
2		SURAJ KARADI	20	M	MIXED	20.7		96	110	70	100.5	14		POSITIVE
3		MALIKARJUN PATIL	20	M	MIXED	21.4		102	100	60	102.9	14		POSITIVE
4		SHRADDHA HERALAGI	25	F	MIXED	24		116	90	60	104.1	16		POSITIVE
5		SUHAS NARAYSWAMI	19	M	VEG	21.9		104	96	60	103.7	18		NEGATIVE
6		ADITYA HOSAMANI	17	M	VEG	24.1		98	106	70	101.1	14		NEGATIVE
7		POOJA BASARAGI	19	F	VEG	21.4		100	104	70	101.6	14		POSITIVE
8		SACHIN ERADATTI	24	M	VEG	23.3		82	120	70	100.8	16		POSITIVE
9		TEJASWINI DHADEKAR	16	F	MIXED	20.8		110	100	58	103.7	16		POSITIVE
10		LAXMAN HONAGOND	25	M	MIXED	25.4		78	118	80	100.1	15		NEGATIVE
11		RATNAMMA GOUDAR	32	F	MIXED	23.4		86	130	80	100.2	12		NEGATIVE
12		NITHIN PUJARI	15	M	VEG	17.9		92	108	60	100.8	16		POSITIVE
13		ABHIJIT BASAVRAJ	17	M	MIXED	17.4		100	110	70	101.4	14		POSITIVE
14		SUNITA RATHOD	38	F	MIXED	23.1		78	140	90	100	16		NEGATIVE
15		BHARAT RAJPUROHIT	18	M	VEG	21.7		98	110	70	100.2	14		POSITIVE
16		PRADEEP BASANT	48	M	VEG	25.3		90	114	60	100.1	16		POSITIVE
17		PALLAVI KANKANWADI	17	F	VEG	23.8		118	100	60	103.5	14		NEGATIVE
18		SUDEEP BIRADAR	16	M	MIXED	20.1		124	90	60	103.8	12		POSITIVE
19		KAVYA MEDEDAR	18	F	MIXED	23.5		84	120	80	100	16		POSITIVE
20		SUSHMITA PATIL	23	F	MIXED	23.4		86	130	80	101.2	18		POSITIVE
21		KIRUTHIGA	22	F	MIXED	23.9		102	112	76	100.5	14		NEGATIVE
22		AKSHAY VISHWNATH	25	M	VEG	26.5		90	110	60	100.7	15		NEGATIVE
23		RAMESH HIREGAN	45	M	VEG	24.8		84	112	70	101.9	12		NEGATIVE
24		ASHOK GHATTARAGI	45	M	MIXED	23.8		88	122	80	101	16		POSITIVE
25		SARFUDDEN	16	M	MIXED	21.9		80	140	80	100.4	14		POSITIVE
26		SERANAV	17	M	VEG	22.9		90	104	60	103.8	16		POSITIVE
27		KALLAWA	32	F	MIXED	23.9		78	122	80	100.5	15		NEGATIVE
28		ABHILASH	26	M	MIXED	23.5		86	124	80	100.4	14		NEGATIVE
29		SHASHIDHAR KULKARNI	17	M	MIXED	18.9		98	110	70	102.8	16		NEGATIVE
30		RAMESH MANTUR	35	M	MIXED	26.3		82	130	74	100.2	14		NEGATIVE
31		SHRISHAIL MAMADPUR	37	M	VEG	27.2		70	110	70	101.2	13		POSITIVE
32		TEJASWINI WALAGED	19	F	MIXED	24.1		92	110	80	102.9	16		POSITIVE
33		SAVITRI PATIL	22	F	MIXED	22.9		96	122	80	100.2	14		NEGATIVE
34		SHEELA EDAGE	20	F	VEG	23.4		100	98	60	102.3	16		POSITIVE
35		VAISHALI NAGANUR	15	F	VEG	17		84	120	70	100.2	14		POSITIVE
36		ROHAN MAHIDRAKAR	15	M	VEG	19		108	100	60	103.3	16		POSITIVE
37		SAVITA PUJARI	22	F	VEG	24.8		120	90	60	102.8	16		POSITIVE
38		MAHADEVAPPA TALVAR	17	M	MIXED	23.5		110	108	70	100.9	14		POSITIVE
39		AKSHATA MURTIGERI	16	F	VEG	19.3		88	110	70	101.6	16		NEGATIVE
40		BORAMMA BIRADAR	16	F	MIXED	18.4		92	120	60	100.6	12		POSITIVE
41		DILIJAN ANASERI	34	M	MIXED	26.8		82	130	80	100.8	13		POSITIVE
42		HARENDRA VERMA	23	M	VEG	22.9		114	100	60	103.3	16		POSITIVE
43		SHARDA MANAMI	32	F	VEG	25		82	110	60	102.2	16		NEGATIVE

44	SUGLABAI	60	F	VEG	22.1	120	90	60	103.8	18	POSITIVE
45	PRASHANT GUDDEDAVAR	32	M	MIXED	26.5	96	140	80	100.1	12	POSITIVE
46	SHANTABAI CHORAGASTI	22	F	MIXED	23.8	102	100	78	102.5	16	POSITIVE
47	MALLIKARJUN	19	M	VEG	21.9	98	110	70	101.2	14	POSITIVE
48	LAXMIBAI SOMANAL	45	F	VEG	24.9	85	130	70	102.2	15	NEGATIVE
49	PRERNA KUMAR	18	F	MIXED	21.7	94	118	70	101	13	POSITIVE
50	JOLIET CYRIL	66	F	VEG	22.1	104	102	66	102.8	17	POSITIVE
51	SRISHAIL KAKKALMALI	18	M	MIXED	24	74	116	70	100.4	15	POSITIVE
52	SHRIDHAR TAKKALKI	16	M	VEG	21.3	92	122	70	99.8	14	NEGATIVE
53	DYAMAWWA	22	F	VEG	23.4	84	110	74	100.2	14	POSITIVE
54	MAHESH BAVACHI	22	M	VEG	22.5	102	100	64	103.2	18	POSITIVE
55	KAVERI BORUTAGI	20	F	VEG	19.3	80	134	70	100.8	14	POSITIVE
56	MALLANNA CHOUDHRI	46	M	MIXED	26.6	88	130	80	100.1	15	NEGATIVE
57	LAXMIBAI RATHOD	58	F	MIXED	18.4	114	100	70	103.1	17	POSITIVE
58	MANISHA RATHOD	19	F	MIXED	21.4	86	120	84	99.9	14	NEGATIVE
59	MANJUNATH YELAGER	19	M	VEG	22.1	98	100	60	102.2	16	POSITIVE
60	GEETANJALI DEDAMANI	17	F	VEG	21.9	90	112	70	101.8	17	POSITIVE
61	SNEHA	23	F	VEG	23.2	86	110	70	101.2	19	POSITIVE
62	SUNIL KUMAR	38	M	MIXED	26.3	78	124	80	100.5	18	POSITIVE
63											