AETIOLOGY OF ACUTE UNDIFFERENTIATED FEBRILE ILLNESS IN ADULT PATIENTS IN A TERTIARY CARE HOPITAL

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BONAFIDE CERTIFICATE

This is to certify that this dissertation work entitled **"AETIOLOGY OF ACUTE UNDIFFERENTIATED FEBRILE ILLNESS IN ADULT PATIENTS IN A TERTIARY CARE HOPITAL"** is the original bonafide work done by **DR.R.THIRUMURUGAN,** Post Graduate Student, Institute of Microbiology, Madras Medical College, Chennai under our direct supervision and guidance.

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Guide & Supervisor sign with Seal.

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Introduction

INTRODUCTION

Acute undifferentiated febrile illness (AUFI) defined as acute onset of fever less than 14 days of duration without any evidence of organ or systemic specific aetiology[5]. No cause found after full history and physical examination [1]. AUFI also known as short febrile illness or acute fever or acute febrile illness. Acute undifferentiated febrile illness different from "pyrexia of unknown origin" in which fever persist for longer duration most probably more than three weeks[2]. Climate variation over population and urbanization may all contribute to the emergence and reemergence of infections in tropical regions. (singh et al, 2012)[10].

The aetiologies of acute febrile illness can vary region wise in India suggesting that diagnosis, treatment, and control programs need to be based on a methodical evaluation of area specific etiologies[11]. Knowledge of local prevalence of infections is essential for areas. The commonest causes include malaria, chikungunya, dengue, scrub typhus, leptospirosis, typhoid fever.

The great diversity of AUFI etiologies are a challenge of diagnosis, treatment and public health responses to endemic and epidemic disease. Given confusion in distinguishing between AUFI, inappropriate use of antibiotics is rampant, frequently by improperly interpreted tests[9].

The lacking of proper diagnostic tools are usually unable to determine specific aetiologies often diagnosing patients presumptively based on clinical features and regarding causative organisms. Clinical features can help in syndrome diagnosis of AUFI [(6)].

Early diagnosis and management of acute undifferentiated febrile illness are necessary as delay in diagnosis and appropriate antibiotic administration can lead to increased mortality[(10)]. Infectious diseases are leading causes of mortality and death in topical countries. The World Health Organisation (WHO) reports that each of the main infectious aetiologies cause between 1.05 and 0.24 million deaths respectively per year in low income. Countries[(7)]. In resource limited settings fever may be treated empirically or self treated due to lack of access to diagnostic tests[(12)].

Number of bacteria, viruses, protozoa and rickettsiae can cause AUFI.

It is important to maintain a proper epidemiological data of AUFI so that evidence based diagnostic criteria and treatment guidelines can be developed. . Health care providers lacking proper diagnostic tools are usually unable to determine specific etiologies often diagnosing patients basted on clinical features.(1) Further confounding that is the fact that a majority of patients present with The management of AUFI based on

scientific rationale, logic, and prevalent clinical practices. Pretest probability of infectious diseases, severity of febrile illnesses availability of specific diagnostics, and response to drug therapy guide to the management. History and physical examination, the traditional tools used by health worker can provide important clues for the etiology of AUFI

Disease burden of infectious etiologies of acute febrile illness is under reported in various parts of India[24].

Syndrome based disease surveillance provides a useful methodology to systematically identify and document causes of acute fever. This approach has been used by European Network for Tropical Medicine[9] and Travel health, to diagnose fevers of Turkey[10], China[11] and India.

However, in Southeast Asian countries limited data exist on the etiologies of AFI in the India. It is important to maintain a proper epidemiological data of AFI so that evidence based diagnostic criteria and treatment guidelines can be developed [24].

Hence this study was undertaken to find out the etiology of AUFI and guide investigations and early treatment of adult patients presenting with fever to internal medicine department in a TERTIARY CARE HOSPITAL .

Aims & Objectives

AIMS AND OBJECTIVES

1. To study the etiology of Acute undifferentiated febrile illness among the fever patients
2. To compare the laboratory parameters with clinical signs and symptoms

Review of Literature

REVIEW OF LITERATURE

Acute undifferentiated fever (AUF) is a common cause of patients seeking healthcare in India, especially between June and September[27]. Unlike fever of unknown origin (FUO), which enjoys a standard definition, AUF, also known as “acute febrile illness”, “short febrile illness”, or “acute fever” lacks an international consensus definition. Since FUO requires duration of fever to be longer than 3 weeks, some authors have defined AUFI as fever that resolves within 3 weeks. More traditionally however, AUF has been defined as fever of 2 weeks or shorter in duration[12]. Thus the term AUF is used to denote fevers that typically do not extend beyond a fortnight, and lack localizable or organ specific clinical features. AUF poses a diagnostic and therapeutic challenge to the health workers, particularly in limited resource setting[14,15]. A number of viruses, bacteria, protozoa and rickettsiae can cause FUO. The non-specificity of symptoms and signs and lack of availability of accurate diagnostics not only test the clinical mettle of even astute physicians but often leads to irrational use of antibiotics and anti-malarials[5]. These syndromes have better developed guidelines for their management. On the other hand, AUF-syndromes (such as fever-rash, fever-myalgia, fever-arthralgia, fever-hemorrhage and fever-jaundice) have overlapping etiologies, which makes their diagnosis and management even more challenging. Fevers with proven diagnosis are known as diagnosed-

AUFIs; those that diagnosis are called undiagnosed undifferentiated fevers UUF[6]. Many UUFs often resolve either on their own or in response to empiric therapies. Diagnosis of many etiologies of AUF in the tropics can be established with help of simple tests, such as peripheral smear examination or rapid diagnostic tests (RDTs) for malaria or dengue. Some other etiologies need more sophiscated tests such as ELISA for rickettsial infections, MAT or ELISA for leptosporosis[5]. Studies that have assessed the cause of AUF in Asia have indicated that depending on the nature of available laboratory support, between a quarter and half of AUFs may remain undiagnosed[14].

Febrile illness can be localized to organ systems or non-localized. There is a paucity of literature on the appropriate evaluation of adult fever patients without localizing symptoms in the emergency medicine department[6]. Acute febrile illness (AFI), the initial diagnosis of whose cause is often presumptive, can sometimes be a challenge for the treating physician. A definite seasonal trend is observed with a peak in incidence with the arrival of the monsoon[13].

So public awareness regarding fevers in the pre-monsoon season should be done. Special care should be given to the elderly as they are often the most vulnerable.

Types of Fever:

Fever is generally classified clinically under the following types:

- Continuous fever – Temperature remains above normal throughout the day and does not fluctuate more than 1 deg c in 24 hours e.g. lobar pneumonia, typhoid, meningitis, urinary tract infection, brucellosis & typhus. Typhoid fever may show a specific fever pattern with a slow stepwise increase and a high plateau.
- Intermittent fever – The temperature elevation is present only for a certain period, later cycling back to normal, e.g. malaria, kala-azar, pyaemia, or septicaemia.

Following are its types:

1) Quotidian fever, with a periodicity of 24 hours, typical of Plasmodium falciparum or Plasmodium knowelesi malaria.
2) Tertian fever (48-hour periodicity), typical of Plasmodium vivax or Plasmodium ovale malaria.
3) Quartan fever (72-hours periodicity), typical of Plasmodium malariae malaria.
4) Remittent fever: Temperature remains above normal throughout the day and fluctuates more than 1 deg c in 24 hours, e.g., infective endocarditis.[47]

SPECIAL TYPES OF FEVER

1. Fever with rigors: This occurs in:
    - Malaria
    - Kala azar
    - Filariasis
    - Urinary tract infection
    - Cholangitis
    - Septicemia
    - Infective endocarditis

2. Fever with herpes labialis: Elevated body temperature may activate the herpes simplex virus and cause small vesicles around the angle of the mouth (herpes labialis). It occurs with:
    - Pneumonia
    - Malaria
    - Meningitis
    - Streptococcal infection

3. Fever with rash: This is seen in:
    - Chicken pox
    - Small pox
    - Measles

- Rubella
- Typhus
- Allergy

4. Fever with membrane in the throat: occurs in:
    - Diphtheria
    - Infectious mononucleosis
    - Agranulocytosis
    - Moniliasis
    - Vincent’s angina.

5. Fever with delirium: This is common in:
    - Encephalitis
    - Typhoid state
    - Meningitis
    - Pneumonia [47]

Pathogenesis:

Fever appears to have evolved on vertebrate hosts as an adaptive mechanism for controlling infection. This phenomenon is produced by certain exogenous (largely microbial) stimuli that activate bone-marrow-derived phagocytes to release a fever-inducing hormone (endogenous

pyrogen). Endogenous pyrogen, in turn, circulates to the thermoregulatory center of the brain (pre-optic area of the anterior hypothalamus) where it causes an elevation in the “set-point” for normal body temperature. Warm blooded animals produce fever by increasing heat production (through shivering) or reducing heat loss (by peripheral vasoconstriction), whereas cold blooded animals do so only by behavioural mechanisms (seeking a warmer environment).

Temperature is ultimately regulated in the hypothalamus. A trigger of the fever, called a pyrogen, causes a release of prostaglandin E2 (PGE2). PGE2 then in turn acts on the hypothalamus, which generates a systemic response back to the rest of the body, causing heat-creating effects to match a new temperature level.

In many respects, the hypothalamus works like a thermostat. When the set point is raised, the body increases its temperature through both active generation of heat and retention of heat. Peripheral vasoconstriction both reduces heat loss through the skin and causes the person to feel cold. If these measures are insufficient to make the blood temperature in the brain match the new set point in the hypothalamus, then shivering begins in order to use muscle movements to produce more heat. When the hypothalamic set point moves back to baseline either spontaneously or with medication, the reverse

of these processes (vasodilatation, and of shivering and non-shivering heat production) and sweating are used to cool the body to the new, lower setting.

CAUSATIVE AGENTS:

AUFI generally has several etiological factors. Few pathogens that are suspected to be etiological agents of AUFI are.

1. MALARIA:

Definition [37,47]

Malaria is a protozoal disease caused by infection with parasites of genus. Plasmodium and transmitted to man by certain species of infected female anopheline mosquito.

There are three stages:-

- Cold stage
- Hot stage
- Sweating stage.

Epidemiology: [51]

Malaria in priority areas

- Forests
- Forested food hills
- Forest fring areas
- Development of project areas

1. Tribal malaria
2. Rural malaria
3. Urban malaria
4. Border malaria.
    a) Plasmodium vivax causes benign tertian malaria
    b) Plasmodium falciparum causes malignant tertian malaria
    c) Plasmodium malariae causes benign quartan malaria
    d) Plasmodium ovale causes tertian malaria.[66]

Life Cycle:

The malarial parasite posses its Life Cycle in two different hosts.

1. In man: The parasite residing inside the liver cell and Red Blood Corpuscle reproduces by asexual method (schizogony). Man represents intermediate host.
2. In female anopheline mosquito:

The sexual forms (male and female gamatocytes) of cycle developed inside the human host. These are transformed to their insect host, where they develop further and transformed into sporozoites. These sporozoites are the infective to man. The mosquito represents the definitive host of the malarial parasite.

Out of 45 species of anopheline mosquitoes in India, only few are as vector of primary importance.[16]

Mode of transmission:

Men gets infection by the bite of female anopheles mosquito. Sporozoites from the salivary gland of the mosquito are directly introduced into the blood circulation.

INCUBATION PERIOD:

P.vivax-8 days

P.falciparum-15 days

P. malariae-13 days

P. ovale-9 days

Clinical features:

COLD STAGE:

The onset is with lassitude, headache, nausea and chilly sensation followed in an hour or so by rigors. The temperature rises rapidly to 39-41°C. Headache is often severe and commonly there is vomiting. In early part of this stage, skin feels cold; later it becomes hot. Parasites are usually demonstrable in the blood. The pulse is rapid and may be weak. This stage lasts for 1/4-1 hour.

HOT STAGE:

The patient feels burning hot and casts off his clothes. The skin is hot and dry to touch. Headache is intense but nausea commonly diminishes. The pulse is full and respiration rapid. This stage lasts for 2 to 6 hours.

SWEATING STAGE:

Fever comes down with profuse sweating. The temperature drops rapidly to normal and skin is cool and moist. The pulse. Rate becomes slower, patient feels relieved and often falls asleep. This stage lasts for 2-4 hours.[51]

DIAGNOSIS:

The diagnosis of malaria depends on demonstration of the parasite in the blood. Suspicion of the diagnosis is aroused by epidemiological and clinical evidence.

A. Two types of blood films are useful in searching for and identification of malaria parasite. The “thin film” and the “thick film”. It is recommended that both types of film be prepared on a single microscope glass slide. The thick film is more reliable in searching for parasite, as large volume of blood is examined under each microscope field. When scanty, parasite may be found about 20 times more rapidly in thick slide than in thin slide. The thin slide is more valuable for

identifying the species of the parasite present. In it they are seen more clearly[16].

B. One step malaria antigen rapid test[43]

TREATMENT[45,51]

Uncomplicated malaria

CHLOROQUINE

Chloroquine base		
Day	**Children**	**Adults**
Day 1	10mg/kg	600mg
Day 2	10mg/kg	600mg
Day 3	5mg/kg	300mg

CHLOROQUINE TABLETS AS PER AGE GROUPS

Chloroquinte tablets 150 mg base			
Age in years	Day 1	Day 2	Day 3
<1	1\2	1\2	1\4
1-4	1	1	1\2
5-8	2	2	1
9-14	3	3	1 1\2
15 & above	4	4	2

PRIMAQUINE P. FALCIPARUMN (51)

Primaquine single dose-day 1		
Age in years	mg base	No. of Tablets (7.5 mg base)
<1	Nil	Nil
1-4	7.5	1
5-8	15	2
9-14	30	4
15 & above	45	6

Malaria vaccines: [51]

Vaccine developed in Colombia (SPf66) advanced to phase III trials in Africa but failed to show efficacy in children under one year old, the highest risk group. Another vaccine (RTS, S/AS02) with the potential to prevent infection and/or ameliorate disease is being tested by GlaxoSmithKline and the MVI at PATH in Phase I trials in children in the Gambia. Beginning in 2002, Phase Ii trials of the vaccine are being conducted among children in Mozambique, which suffers from year-round malaria transmission-offering a better opportunity to evaluate vaccine performance [46].

2. DENGUE[66]

Definition:-

Dengue fever is zoonotic illness caused by viruses. Dengue viruses are arboviruses capable of infecting humans and causing disease. Arboviruses are group of ssRNA viruses. These infections may be asymptomatic or may lead to

a) "classical" dengue fever
b) Dengue haemorrhagic fever
c) Dengue shock syndrome.

Vectors:

Blood socking arthropods (Insect Vectors). Aedes aegupti is the principal vector. They bite during the day time. Aedes aegupti is nervous feeder and most efficient vector. Incubation period is 8-10 days. Once infected, it remains infective for life. Aedes can pass the dengue virus to the off spring by transovarial transmission.

CLINICAL FEATURES AND DIAGNOSIS:

a) Dengue fever (DF)
b) Dengue haemorrhagic fever (DHF)
c) Dengue shock syndrome (DSS)

CLINICAL DIAGNOSIS: Of DHF without shock

a) Fever – acute onset, high, continous and lasting 2 to 7 days.

b) Haemorrhagic manifestations

- Positive tourniquet test and
- Petechiae, purpura, ecchymosis
- Epitaxis, gum bleeding
- Haematamesis/ melena.

c) Hepatomegaly

Grading of severity of DHF [65]

- Grade I : Fever accompanied by non-specific constitutional symptoms. Positive tourniquet test.
- Grade II: Patient with spontaneous bleeding usually in the form of skin and other haemorrhages in addition the grade-I
- Grade III: circulatory failure by rapid pulse and narrowing pulse pressure or hypotension with presence of cold clammy skin and restlessness.
- Grade IV: Profound shock with undetectable blood pressure and pulse.

Laboratory diagnosis:

Thrombocytopenia (100,000/mm³ or less). There was a significant correlation of IgG titres with platelet counts, with higher titres associated with lower platelet counts. It is speculated that IgG, IgA and IgE produced in response to primary infections.

- NS1 Antigen
- IgM capture ELISA

TREATMENT

The management of dengue fever is symptomatic and supportive. Bed rest is advisable during the acute febrile phase. Antipyretics or sponging are required to keep the body temperature below 40°C. The management of DHF during the febrile phase is similar to that of DF> A rise in haematocrit value indicates significant plasma loss and a need for parenteral fluid therapy. Serial haematocrit determination, every four to six hours, and frequent recording of vital signs are recommended for adjusting the fluid replacement in order to assure adequate volume replacement and avoid over transfusion.

Crystelloid: five per cent dextrose in lactated Ringer's solution, fiver per cent dextrose in acetated Ringer's solution, five per cent dextrose in half strength normal saline solution and fiver per cent dextrose in normal saline solution. Colloidal: Dextran 40 and plasma.[49]

MANAGEMENT OF SHOCK:

DSS is a medical emergency that requires prompt and vigorous volume replacement therapy. There are also electrolyte (sodium) and acid-base disturbances. It must be considered that there is a high potential for developing disseminated intravascular clotting (DIC) and that stagnant acidaemia blood will promote and/or enhance DIC, which may lead to severe haemorrhage and/or irreversible shock [47,51].

REPLACEMENT OF PLASMA LOSS:

Blood transfusion is indicated in cases with profound or persistent shock despite declining haematocrit values after initial fluid replacement.

CONTROL MEASURES

1. Mosquito control:

The vectors of DF and DHF (e.g., A. aegypti) breed in and around houses and, in principle can be controlled by individual and community action, using antiadult and antilarval measures. These measures are outlined.

2. Vaccines:

So far, there is no satisfactory vaccine and no immediate prospect of preventing the disease by immunization.

3. CHIKUNGUNYA:

Definition:

Chikungunya is a dengue – like disease caused by a group virus, and transmitted by the genus Aedes, culex, monsonia mosquitoes.

Chikungunya virus belongs to

- Family – togaviridae
- Genus – Alpha virus

Based on clinical manifestations it can be catagorised into fever-arthiritis group. Chikungunya fever is a re-emerging disease characterized by acute fever with arthalgia.

Transmission:

Transmitted by Aedes aegupti mosquito which bite during day time. Rarely by vertical transmission from mother to fetus or by blood transfusion[16].

CLINICAL FEATURES

Incubation period 4-7 days

- Sudden onset of fever, chills
- Anorexia
- Lumbago

- Conjunctivitis
- Morbiliform rashes with purpura on the trunk and limbs

Symptoms:

- Coffee coloured vomiting
- Epitaxis
- Petechiae
- Arthropathy- excruciating joint pains, swelling and stiffness especially in wrist, elbow, shoulder, knee ankle and metatarsal joints. It can be persists for months and even years.

Diagnosis:

Virus can be isolated from the blood of febrile patients by the intracerebral inoculation in suckling mice or on VERO cells.

Serdogical diagnosis:

ELISA is detected for IgM antibody.

Treatment:

There is no specific treatment of chikungunya infection and it is usually self limiting. Analgesics, antipyretics like paracetamol, diclofenac sodium, chloroquine along with fluid supplementation are recommended to manage infection and relieve fever, joint pains and swelling [37,47].

CONTROL:

Vector control[52]

- Keep water storage free of mosquitoes and eliminate the other breeding places in and around the houses.
- The organophospharous insecticide, Abate is used as a larvicide.
- Anti adult measures can bring about a rapid interruption of transmission.
- A new technique consisting of aerosol spray of ultra low-volume (ULU) quantities of malathion or sumithion is effective in stopping the transmission. (250ml/hectre)

4. LEPTOSPIROSIS:

Leptospirosis is the most widespread of the disease transmited from animal to man. Out breaks occur due to season of heavy rain fall and consequent floodings[47,53-55].

Epidemiology:

AGENT FACTORS:

Leptospira are thin and light motile spirocheates 0.1-0.2µm wide and 5-15 µm long with hooked ends. Leptospira interrogans are pathogenic. The organisms are visible by clark-field illumination and silver staining[17].

SOURCE OF RESERVOIRS:

Leptospirosis affects wild and domestic animals worldwide especially rodents such as rats, mice, and voles. [45]

MODE OF TRANSMISSION:

a) Direct contact

b) Indirect contact

c) Droplet infection

INCUBATION PERIOD:

Usually 10 days with a range from 4-20 days.

CLINICAL FEATURES:

Leptospirosis can manifest in many ways. The various syndromes of presentation are as follows:

1. Anicteric leptospirosis
2. Icteric leptospirosis (weil's disease)
3. Haemorrhagic fever with renal syndrome.
4. A typical pneumonia syndrome.
5. Myocarditis
6. Ocular manifestations. [47]

INCUBATION PERIOD:

4 to 20 days.

DIAGNOSIS:

IgM ELISA is useful in making an early diagnosis.

CONTROL:

Antibiotics:

Penicillin is the drug of choice but other antibiotic (tetracycline or doxycycline) are also effective. The dosage of penicillin 6 million units daily intravenously.

Environmental factors:

Preventing the exposure contaminated water reducing the contamination by rodent control and protection of workers in hazardous occupation. Measures should be taken to control rodents proper disposable of wastes and health education. [53]

Vaccination:

Vaccines are available. Immunization of farmers and pets prevent disease. Immunity to one type of leptospira may not protect against infection by another type[57].

5. SCRUB TYPHUS:

Scrub typhus is caused by orientia tsutsugamushi. It differs from rickettsiae genetically as well as by cell wall composition (It lacks of polysaccharide layer) [26].

AGENT:

The causative agent of scrub typhus is Orientia tsutsugamushi.

Reservoir:

The true reservoir of infection is the trombiculid mite.

- Leptrombidium delinese
- Leptrombidium akamushi
- The nympal and adult stages of the mite are free living in the soil.
- Chiggerosis: Among all stages of mite, the larva (chiggers) are the only stage that feed on humans. That is called chiggerosis.
- Mites can maintain the organisms through transovarian transmission.[47]

Mode of transmission:

The mite of infected larval mites. The transmission cycle as below.

Mite->Rats and mice->mite->Rats and mice

The disease is not directly transmitted from person to person.

INCUBATION PERIOD:

10-12 days: varies 6-12 days.

Clinical features:

- Acute fever with chills (104°C-105°F)
- Headache /myalgia
- Malaise/cough
- Prostration/GIT symptoms
- Macular rash-5th day of illness.
- Generalized lymphadenopathy and lymphocytosis[17]

Punched out ulcer covered with a blackened scab (eschar) which indicates the location of the mite bite.

Laboratory test:

- One step, scrub typhus Antibody test(Immuno chromatographic test).

Treatment:

Tetracycline is the drug of choice. [4]

Control measures:

Cleaning the vegetation where rats and mite live. Application of insecticides such as lindane or chlordane to the ground .

- Personal prophylaxis. [51]

6. TYPHOID FEVER:

Typhoid fever is the result of systemic infection mainly by salmonella typhoid found only in man.

The term “enteric fever” includes both typhoid and paratyphoid fevers. The disease may occur sporadically, epidemically or endemically[47].

AGENT FACTORS:

Salmonella typhi is the major cause of enteric fever. Salmonella paratyphi A, paratyphi B are relatively infrequent. Salmonella typhi has three main antigen –o, H, and vi.

RESERVOIR OF INFECTION:

Man is the only known reservoir of infection through cases and carriers.

(i) Cases may be mild or severe carrier is infectious as long as bacilli appear in stool or urine.

(ii) CARRIERS: The carriers may be temporary or chronic.

Clinical features

The classic presentation includes fever, malaise, diffuse abdominal pain, and constipation. Untreated, typhoid fever is a grueling illness that may progress to delirium, obtundation, intestinal hemorrhage, bowel perforation, and death within 1 month of onset. Survivors may be left with long-term or permanent neuropsychiatric complications[(58)].

Typhi has been a major human pathogen for thousands of years, thriving in conditions of poor sanitation, crowding, and social chaos. In the advanced stages of typhoid fever, the patient's level of consciousness is truly clouded. Although antibiotics have markedly reduced the frequency of typhoid fever in the developed world, it remains endemic in developing countries(59).

INCUBATION PERIOD: 10-14 days.

MODE OF TRANSMISSION:

Typhoid fever transmitted through the faecal-oral roate or urine-oral route.

TREATMENT[47]:

	Drug of choice	Alternate drugs
Emperical Treatment	This is the treatment given before antimicrobial susceptibility report is available. Ceftriaxone	Azithromycin.
Fully susceptible	Ciprofloxacin	Amoxycillin chloramphenical cotrimoxozole
MDR strains (multidrug resistant)	Ciprofloxacin	Ceftriaxone Azithromycin.
NAR strains (NALIDIXIC acid resistant)	Ceftriaxone	Azithromycin. Ciprofloxacin
Carriers	Ampicillin or Amoxycillin probenecid for 6 weeks	Cotrimoxozole or Ciprofloxacin.

CONTROL OF TYPHOID FEVER:

There are three methods of defence against typhoid fever.

a) Control of reservoir

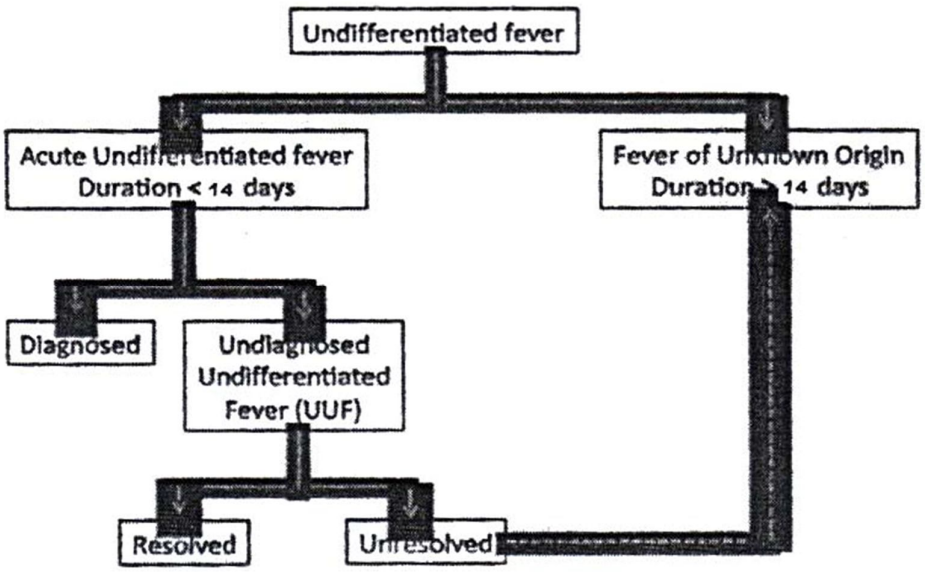
b) Control of sanitation

c) Immunization[47,60]

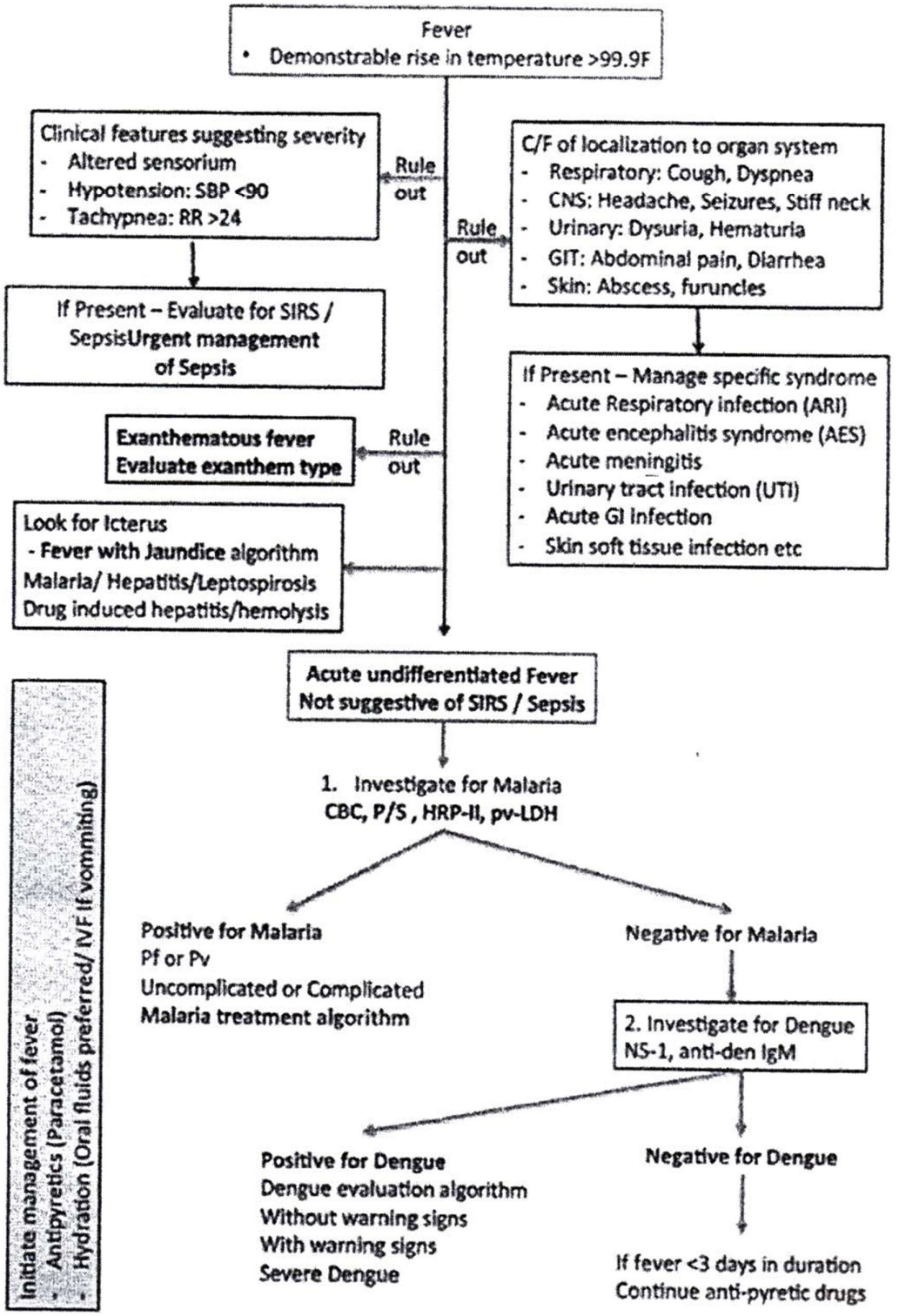
VACCINE:

1. Parentral TAB vaccine.
2. Parentral V1 polysaccaride vaccine
3. Typhoid (oral live attenucated s. TyphiiTyz Ia Vaccine [47,55,58]

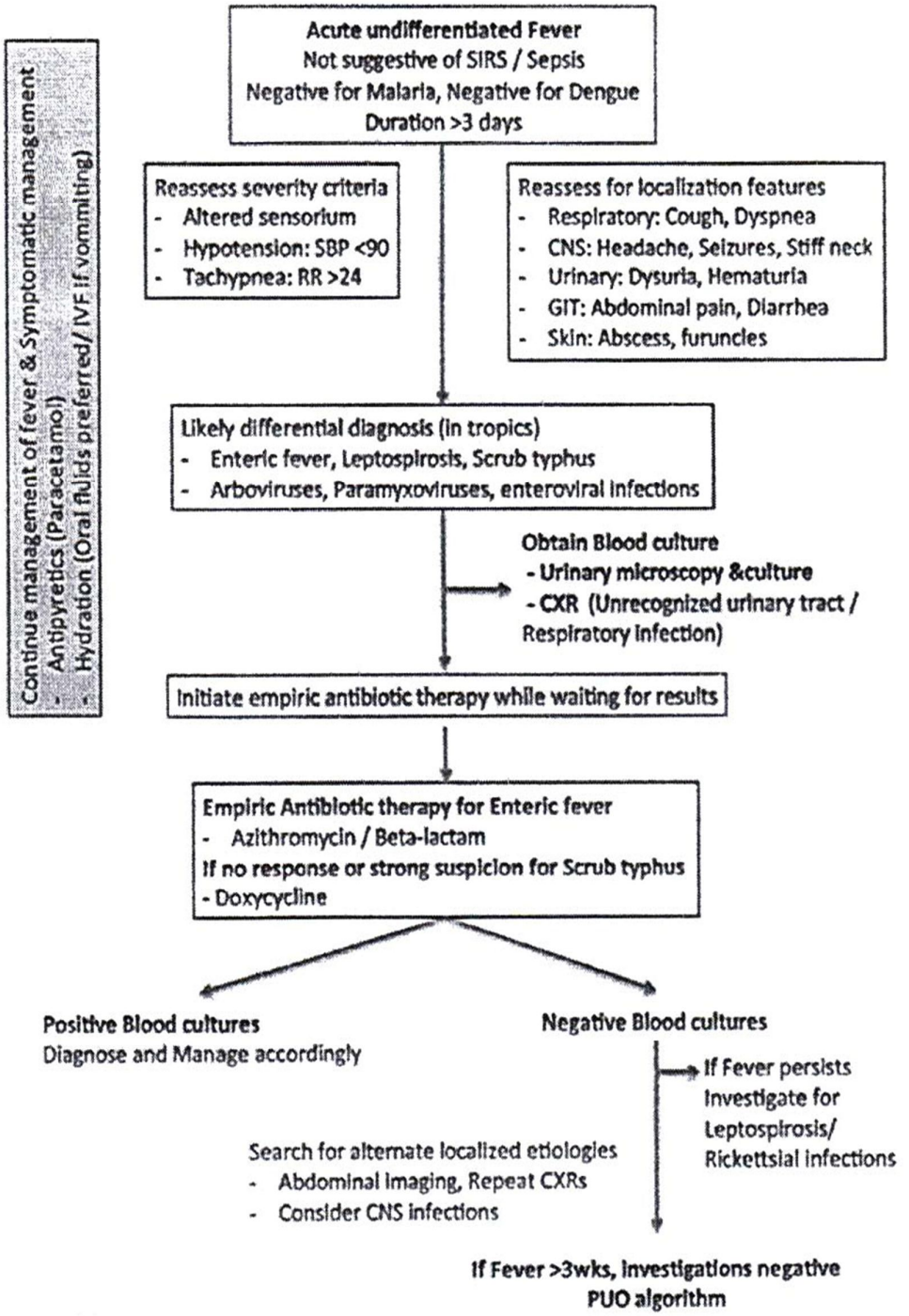
DIAGNOSIS OF FEVER:



Initial steps in assessment of Acute Undifferentiated fever



Assessment of Acute Undifferentiated fever when initial RDTs are negative



Materials & Methods

MATERIALS AND METHODS

Place of study:

Institute of Microbiology & Institute of Internal Medicine,

Rajiv Gandhi Government General Hospital (RGGGH),

Chennai – 03.

Study design:

Prospective study.

Study period:

One year.

Sample size:

150 patients presented with AUFI admitted in a tertiary care hospital.

Ethical consideration:

All patients detail, satisfying the following inclusion criteria will be documented and taken up for the study after obtaining informed written concern in both regional language and English. This study was reviewed by Institutional Ethics Committee and clearance number obtained.

Inclusion criteria:

- Adult patients of more than 18 years of age
- Patients with acute fever less than 14 days duration with body temperature of more than 38 deg C who are admitted in the hospital.
- The cause of illness undiagnosed after medical history and physical examination.

Exclusion criteria:

- Patients aged less than 18 years.
- Fever more than 14 days
- Patient with clinical systemic involvement.
- Patient with coexisting infections.

BLOOD CULTURE:

Sample collection and processing:

Under strict aseptic precautions, venipuncture site was cleared with 70% alcohol and then with 2% poidane Iodine[61]. The disinfectant was allowed to act for 1 minute and then 10ml of blood sample was collected with a sterile syringe and added into a sterile crew capped blood culture bottle containing 50ml of sterile Brain heart infusion broth (BHI) broth. (1:5 dilution) and was labeled with name, age, sex, IP No date and time of collection and culture bottles transported immediately to the laboratory[21].

Sample processing:

The culture bottles were incubated at 37°C for 24 hours. Observe for turbidity, pellicle formation and deposits, the subcultures made on to the following plates using sterile techniques[18].

Interpretation

All inoculated plates were observed for growth after 24 hours. The colony size, shape, edge, margin and consistency was noted.

Subculture was done on NAP, MAC, 5% sheep blood agar plate.

NAP – colonies on these media are 2-3mm in diameter, greyish white, circular, moist, convex and translucent colonies, opaque and granular colonies with irregular surface.

BAP – Non hemolytic moist colonies.

MAC – colonies are round (1-3mm size) translucent, pale and non-lactose fermenting.

Biochemical identification of Salmonellae:

- Catalase - positive and oxidase – negative

ICUT test:

Indole – Negative

Citrate – Positive – salmonella paratyphi-B

Negative – Salmonella Typhi and salmonella paratyphi – A.

Urease – Negative

Triple sugar iron test

- Alkaline / acid
- Gas present (Salmonella typhi – No gas present)
- Abundant H_2S present – salmonella paratyphi B salmonella Typhi speck of H_2S present at the junction of slant and butt.
- MR positive and VP negative.

Decarboxylation test(LAO):

- Salmonella Typhi – only lysine is decarboxylated
- Salmonella paratyphi A – only ornithine
- Salmonella paratyphi B – positive for all [21].

Antimicrobial susceptibility Testing:

Antibiogram was done on Mueller-Hinton agar by disk diffusion method.

Antibiotic Sensitivity pattern. As per CLSI Guidelines [21]. 26th Edition, M100S

Drugs	Disc content	Sensitive (mm)	Intermediate Sensitive (mm)	Resistance (mm)
Ampicillin	10 µg	≥ 17	14-16	≤ 13
Cotrimoxazole (Tmp/smz)	1.25/23.75	≥ 16	11-15	≤ 10
Chloramphenical	30 µg	≥ 18	13-17	≤ 12
Ceftrioxone	30 µg	≥ 23	20-22	≤ 19
Nalidixic acid	30 µg	≥ 19	14-18	≤ 13
Pefloxacin	5µg	≥ 24	13-15	≤ 23
Azithromycin	15µg	≥ 13	-	≤ 12
Cefotoxime	30µg	≥ 26	23-25	≤ 22

Antimicrobial Susceptibility pattern of S.Typhi by disc diffusion method [(63)].

Organism	AMPI 10µg	COT 1.25 / 23.75µg	CK 30µg	CTX 30µg	CFT 30µg	AZIT 15µg	NA 30µg	PEF 5µg
S.Typhi (n=5)	100%	100%	100%	100%	100%	100%	0	0

Ciprofloxacin has remained the drug of choice of enteric fever for last many years. However, isolates of S.Typhi with reduced susceptibility for fluoroquinolones have been appeared in the subcontinent, Vietnam, Tajikistan and the treatment failures with fluoroquinolones has also been noted.

In vivo adaptive mutations have been reported to occur on continuous sub-inhibitory exposure to Ciprofloxacin. Also the frequent use of Pefloxacin for trivial infections is likely to promote resistance to these agents.

PERIPHERAL SMEAR:

The pulp of a finger of the patient was wiped with alcohol & allowed to dry. Then it was picked with surgical cutting needle and two drops of blood was taken on a grease free clean glass slide, one half an inch the right end of the slide. The former was made into a thick film and later into a thin film. The drop for thick film was spread with a needle to form an area on half an inch square. Through this film a news paper print should be read. The film should be prepared by folding a spreader at smooth edge of glass slide an angle of 45° in contact with the drop of blood, then lowered to an angle of 30° and pushed gently to the left till the blood is exhausted to form

tail. The film is allowed to air dry. The thick film is dehaemoglobinised by placing in a glass cylinder with distilled water in a vertical position for 5 to 10 mins. When the film becomes white it was taken out and allowed to dry on upright position. Undiluted Leishman ‘s stain and after dilution on the stain is poured over the thin film is flooded over the thick film. The stain was allowed remain on the slide for 10 to 15 mints. The slide was washed with running tap water and allowed to dry on upright position. Then it was examined under oil immersion lens[16].

SEROLOGICAL TEST:

Blood was collected by aseptic technique. Prior to venipuncture, the skin must be decontaminated with 2% chlorohexidine or 70% alcoholic rub by making a smooth circular pass over the site and moved in an outward spiral from the zone of penetration. The skin was allowed to dry before processing. For each patient about 5ml of blood sample was collected by venipuncture by a disposable sterile syringe and transferred to a sterile labeled test tube. Left to clot for 30mins and then centrifuged at 3000x8 from which the serum was separated, aliquoted and preserved at -70°c[16].

DENGUE NS1 ANTIGEN CAPTURE ELISA:

Principle: Serum dengue NS1 antigen, when present, binds to anti-Ns1 antibodies attached polystyrene surface of the microwells. Residual

serum is removed by washing, and HRP conjugated Anti-NS1 MAb is added. After incubation, the microwells are washed and a colourless substrate system, tetramethylbenzidine\hydrogen peroxide (TMB chromogen) is added. The substrate is hydrolysed by the enzyme and the TMB changes to a blue colour. After stopping the reaction with the acid, the TMB turns yellow. Colour development is indicative of the presence of Dengue NS1 antigen in the test sample.

Test procedure: The patients sample and all the reagents of the kit were brought to room temperature. The required microwells were removed from the foil sachet and inserted into the strip holder. Using suitable test tubes or a microtitre plate the Positive Control, Negative Control, Calibrator and patients sample were diluted. (75µl of sample diluents was added to 75µl of sample and mixed well. The final dilution was 1 in 2.

100 µl of diluted test samples and controls (positive control, Negative control and Calibrator in triplicate) was pippetted into the respective microwells.

- The plate was covered and incubated for one hour at 37°c.
- Washed 6 times with diluted wash buffer.
- 100 µl of HRP conjugated Anti-NS1 MAb was pipette into each well.
- The plate was covered and incubated for one hour at 37°c.

- Washed 6 times with diluted wash buffer.
- 100 µl of TMB pipette into each well. Incubated for 10 minutes at room temperature. A blue colour developed.
- 100 µl of stop solution was pipette into all wells in the same sequence and timing as the TMB addition and mixed well. The blue colour changed to yellow.
- Within 30 minutes the absorbance of each well was read to a wavelength of 450nm with a reference filter of 600-650nm.

QUALITY CONTROL:

- Abs (a) 450nm
- Calibrator factor : 0.69
- Negative Abs : >0.300
- Cut off value : ≥2.0 x neg Abs
- Positive cut off ratio : 1:1 - 7.0

CALCULATIONS:

The average absorbance of the calibrator triplicates was calculated and multiplied by calibration factor. This is the cut-off value. Index value was calculated by dividing the sample absorbance by cut-off value.

Interpretation of results:

- If Index value <0.9 it is negative.
- If Index value is 0.9-1.1 then it is equivocal.
- If Index value is >1.1 it is positive.

DENGUE IgM CAPTURE ELISA:

Principle: serum antibodies of the IgM class, when present binds to anti-human IgM antibodies attached to polystyrene surface of the microwell test strips. A concentrated pool of Dengue 1-4 antigens is diluted to correct working volume with antigen diluents. The antigens are produced using an insect cell expression system and immunopurified utilizing a specific monoclonal antibody. Anequal volume of the HRP conjugated MAb is added to the diluted antigen, which allows the formation of antigen-MAb complexes. Residual serum is removed from the assay plate by washing and complexed antigen MAb is added to the assay plate. After incubation, the microwells are washed and a colourless substrate system, tetramethylbenzidine / hydrogen peroxide (TMB chromogen) is added. The substrate is hydrolysed by the enzyme and the TMB changes to a blue colour. After stopping the reaction with the acid, the TMB turns yellow. Colour development is indicative of the presence of anti-dengue IgM antibodies in the test sample.

TEST PROCEDURE:

The patients sample and all the reagents of the kit were brought to room temperature. The required microwells were removed from. The foil sachet and inserted into the strip holder. Using suitable test tubes or a microtitre plate the positive control, negative control, calibrator and patients sample were diluted. (1000µl of sample diluents was added to 10µl of sample and mixed well.

The antigen was diluted to 1/250 using antigen diluents.

Required volume of diluted antigen was mixed gently with equal volume of MAb Tracer in a clean polypropylene vial and waited for 10 minutes.

100µl of diluted test samples and controls (positive control, Negative control and Calibrator in triplicate) was pippetted into the respective microwells.

- The plate was covered and incubated for one hour at 37°c.
- Washed 6 times with diluted wash buffer.
- 100µl of mixed antigen-MAb tracer was pipette into each well.
- The plate was covered and incubated for one hour at 37°c.
- Washed 6 times with diluted wash buffer.

- 100µl of TMB pipette into each well. Incubated for 10 minutes at room temperature. A blue colour developed.
- 100µl of stop solution was pipette into all wells in the same sequence and timing as the TMB addition and mixed well. The blue colour changed to yellow.

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

QUALITY CONTROL:

- Abs (a) 450nm
- Calibrator factor : 0.80
- Negative Abs : >0.400
- Cut off value : ≥1.5xnegAbs
- Positive cut off ratio : 1:1.9.0

CALCULATIONS:

The average absorbance of the calibrator triplicates was calculated and multiplied by calibration factor. This is the cut-off value.

Index value was calculated by dividing the sample absorbance by cut-off value.

Interpretation of results:

- If Index value < 0.9 it is negative.
- If Index value is 0.9-1.1 then it is equivocal.
- If Index value is >1.1 it is positive.

CHIKUNGUNYA IgM CAPTURE ELISA:

Procedure: Serum was diluted1:!00 in deep well plate using sample diluents for CHIK IgM

Required number of anti IgM coated strips was removed from the pouch.

The strips was washed 3 times with 1x wash buffer and the wells were not allowed to dry.

50µl of the diluted samples were transferred from the deep well plate to respective wells as per the protocol on ELISA sheet using multichannel pipette.

Add 50µl of CHIK IgM Positive control and CHIK IgM negative control to respective wells .(The controls were not diluted)

The plate was covered with aluminium foil and incubated at 37^0C for one hour.

The plate was then washed five times with 1x wash buffer.To remove traces of wash buffer content the plate was tapped on a tissue paper after last wash.

50µl of CHIK antigen was added to each well of the plate.

Again the plate was covered with aluminium foil and incubated at 37^0C for one hour.

The plate was then washed five times with 1x wash buffer.To remove traces of wash buffer content the plate was tapped on a tissue paper after last wash.

- 50µl of Anti CHIK MAbHxB(Biotin labeled) was added to each well.
- Then it was incubated and washed as before.
- 50µl of Avidin-HRP was added to each well.
- The plate was covered with aluminium foil and incubated at 37^0C for 30 minutes.
- Washed 5 times with1x wash buffer as before.
- 100µl of Liquid TMB Substrate (TM/H_2O_2) was added to each well.
- Incubated at room temperature in dark for 10 minutes.

- The reaction was stopped after 10 minutes with addition of 100µl of stop solution.
- The absorbance was measured at 450 n as early as possible.

QUALITY CONTROL:

1. If OD of negative control is more than0.18 or
2. If OD of positive control is less than 6 times the OD of negative control.

   In both the situations, the test should be considered as invalid.

Interpretation of Results:

1. Sample OD ≤ Negative control x 2.0 - sample should be considered as negative
2. Sample OD ≥ Negative control x 3.0 - sample should be considered as positive
3. Sample OD ≥ Negative control x 2.0 but less than Sample OD ≤ Negative control x 3.0-sample should be considered as equivocal.

MSAT (Macroscopic Slide Agglutination Test):

Principle: Slide agglutination test using formalinised and heat killed pooled antigen from leptospires belonging to different serovars commonly prevalent in and around Chennai.

Materials Required:

- Prepared in-house leptospiral antigen
- Cavity slide
- Phosphate buffered saline(PBS) pH7.2
- Test sera
- Micropipette (range:5-50µl)

Procedure:

One drop (12 µl) of antigenic substance was mixed with a drop of PBS(7 µl) and patients sera of about 6 µl on a depression slide and rotated on a rotator at 120 rpm for 8 minutes. It was examined macroscopically for the presence of agglutination and also confirmed using dark field microscope. Positive and negative controls were also put up.

Interpretation:

It was interpreted as follows:

1	Clumps of agglutination with complete clearing of leptospiral antigen suspension	4+
2	Obvious agglutination but partial clearing of suspension	3+
3	50% agglutination	2+
4	25% agglutination	1+
5	No agglutination and uniformity of serum antigen mixture	Negative

A 2+ and above agglutination titre is indicative of recent infection and considered significant.

ONE STEP SCRUB TYPHUS ANTIBODY TEST

The SD Bioline Tsutsugamushi test was developed using the major surface antigen 56 kDa of representative strains of Orentia tsutsugamushi (Karp, Kato and Gilliam).It is solid phase, immunochromatographic assay for the rapid qualitative detection of IgG,IgM or IgA antibodies to O.tsutsugamushi in human serum,plasma or whole blood.

Principle:

This test has 2 pre-coated lines on the surface of the strip.”T(O.tsutsugamushi antibody test line)and “C”(control line.The control line is used for procedural control and should always appear if the test procedure is performed properly and the test reagents of the control line are working.A purple “T “line will be visible in the result window if there are enough IgG,IgM or IgA O.tsutsugamushi antibodies in the specimen.If IgG,IgM or IgA antibodies to O.tsutsugamushi are not present in the specimen,no colored “T“line will appear.

Procedure:

All the kit components and the specimens were brought to room temperature prior to testing.

The test device was removed from the pouch and kept on a flat,dry surface.

10µl of serum was slowly dispensed to the specimen well (S) and then 3-4 drps of assay diluents was dispensed.

As the test begins to work the purple color moved across the result window in the centre of the device.

The test was interpretated at 10-15 minutes.

Interpretation:

A colored control line will appear in the left section of the result window to show that the test is working properly.

Negative result: The presence of only the control line "C" within the result window indicates a negative result.

Positive result: The presence of both the "C"and "T" line within the result window indicates a positive result.

Invalid result : If the “C” line is not visible within the result window after performing the test, the result is considered invalid.

ONE STEP MALARIA ANTIGEN RAPID TEST

The whole blood of 2ml was collected by venipuncture under strict aseptic precaution into the collection tube containing EDTA.If the test was delayed the sample was refrigerated at 2-8◦C for not more than 3 days.

Alere Trueline™ Rapid Test Kit for Malarial Ag P.f/P.(HRP-II/pLDH) Test is a one step,rapid qualitative and differential test for the detection of Histidine Rich Protein-II specific to Plasmodium falciparum and Plasmodium Lactate Dehydrogenase pan specific to Plasmodium species in human blood specimen.

Principle of the Test:

This test cassette contains a membrane strip which is precoated with one monoclonal antibody and one polyclonal antibody as two separate lines across a test strip.One monoclonal antibody (test line P.f) is specific to HRP-II of p.falciparum and other polyclonal antibodies (test line pan) are pan specific to the LDH of Plasmodium species (P.vivax, P.malariae, P.ovale).The test device has “Test lines”(P.f and Pan) and “Control line”on the surface of the device.

Procedure:

The specimen and kit components are brought to room temperature prior to testing.

The test device was removed from the pouch and kept on a flat, dry surface.

5µl of blood specimen was carefully placed into the round sample well using a disposable specimen loop provided.

4 drops of assay diluents was added into the square assay diluent well.

Test results was interpreted within 20-30 minutes.

Interpretation of the results:

Negative result:The presence of only the control line "C" within the result window indicates a negative result.

P.falciparum Positive:The presence of two color bands ("P.f" Test line and "C"control line) or three color bands("P.f","Pan" Test lines and "C"control line) within the result window indicates a positive result.

Other Plasmodium species (P.v,P.m,P.o)positive: The presence of two color bands (“Pan” Test line and “C”control line) within the result window indicates other plasmodium species positive result.

Mixed infection:P.f and P.v (or P.m,P.o): The presence of three color bands(“P.f”,”Pan” Test lines and “C”control line) within the result window may indicate a mixed infection.

Invalid result: If the “C” line is not visible within the result window after performing the test, the result is considered invalid.

WIDAL TEST

Principle of the test: The organisms causing enteric fever possess two major antigens namely somatic 'O'antigen and flagellar 'H' antigen.During infection antibodies are produced in patients sera against these 'O' and 'H' antigens. Widal test detects the amount of antibodies formed in the patients serum.

Method:

Master dilution of the patients serum was prepared in a test tube of 12x100 mm. 0-2. 2.3 ml of saline (0.85% Sodium chloride) was added to 0.2 ml of patients serum.This gave 1:12.5 dilution. Then a test tube rack

having 8-10 holes in 4 rows each was taken. 7 test tubes was kept in each row and labelled as(l) TO (2)TH (3)AH and (4) BH.

From the master dilution,0.2ml was added into the first and second tube in all rows of tubes T0,TH,AH and BH.

0.2ml of saline was added into all tubes from 2-7 in all rows including the controls (7^{th} tube). The dilution of the serum was carried out as follows.0.2ml was mixed and transferred from tube 2 to 3,then from 3 to 4 and so on ,through tube 6.0.2ml was discarded from tube 6.

The same dilution was followed for TH,AH and BH rows. For the rows 'TO'/TH', 'AH', 'BH' 0.2 ml of corresponding 'TO'/TH', 'AH', 'BH' antigen was added into 1-7 tubes of respective rows. The final dilution obtained was l:25,l:50,l:100,l:200,l:400,l:800.Tube 7 in each row was a negative antigen control.

For positive control same dilutions was proceeded with TO, T, H positive serum.

The rack was incubated at 37°C for 18-24 hours and the results were read as follows. "H"Agglutination -formation of loose, cotton wooly clumps. "O"Agglutination-granular matt like spread at the bottom of the

tube. Controls with no agglutination was seen as compact button of settled organisms.

The last tube showing the agglutination was taken as the end point. The reciprocal of that dilution was taken as the titre.

100 µl of mixed antigen - MAb tracer was pipette into each well. The plate was covered and incubated for one hour at 37°c. Washed 6 times with diluted wash buffer.

100µl of TMB pipette into each well. Incubated for 10 minutes at room temperature. A blue colour developed.

100µ1 of stop solution was pipette into all wells in the same sequence and timing as the TMB addition and mixed well. The blue colour changed to yellow.

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

QUALITY CONTROL:

Abs (a) 450nm

Calibrator factor : 0.80

Negative Abs : >0.400

Cut off value : >1.5xnegAbs

Positive cut off ratio : 1:1.9.0

CALCULATIONS:

The average absorbance of the calibrator triplicates was calculated and multiplied by calibration factor. This is the cut-off value.

Index value was calculated by dividing the sample absorbance by cut-off value.

Interpretation of results:

If Index value < 0.9 it is negative.

If Index value is 0.9-1.1 then it is equivocal.

If Index value is >1.1 it is positive

Results

RESULTS

Blood samples were collected from 150 patients who were clinically suspected to have no other definite sources of infections identified such as pneumonia, acute infectious diarrhea, UTI, smear positive pulmonary Tuberculosis, skin and soft tissue infections except fever as the main symptom. The samples collected and processed in microbiology laboratory. The results were analysed and tabulated as follows

Table-1 : AGE WISE DISTRIBUTION (n=150)

Age (Years)	Number	Percentage
18-30	82	54.7%
31-40	33	22%
41-50	18	12%
51-60	6	4%
61-70	10	6.7%
71-80	1	6%
Total	150	100%

AUFI most commonly presenting age groups are in between the 18-30 years

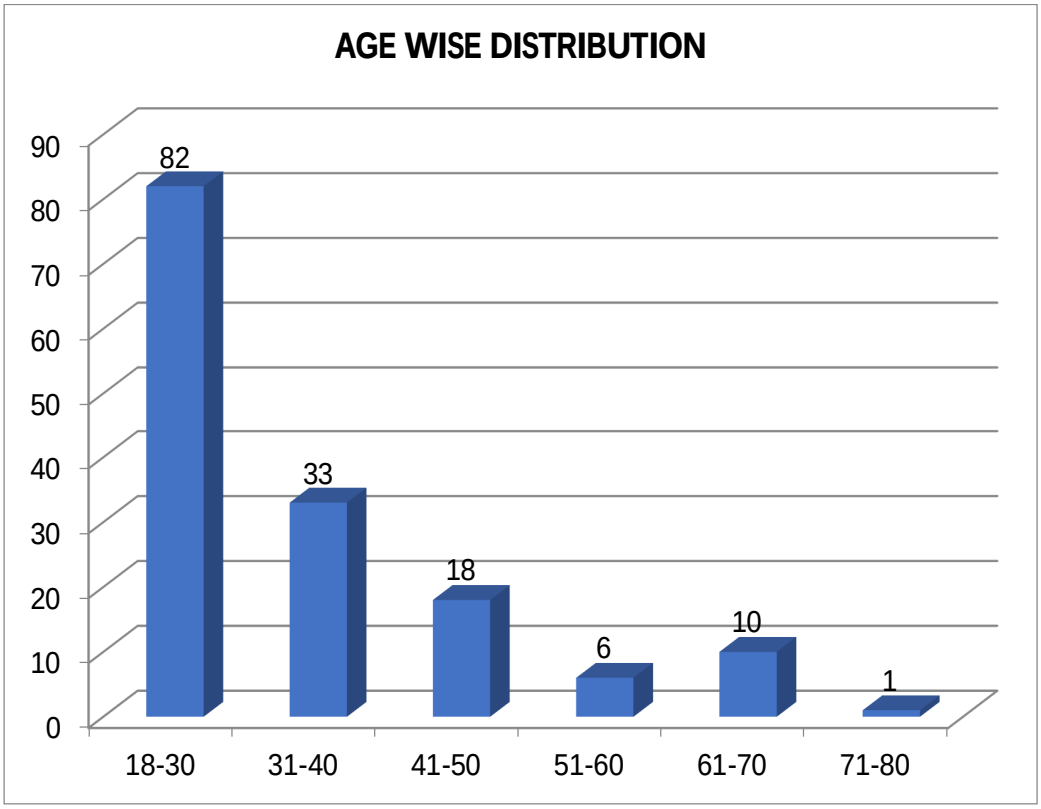


Table – 2 : GENDER WISE DISTRIBUTION (n=150)

Sex	Number	Percentage
Male	85	57%
Female	65	43%
Total	150	100%

The males are more commonly affected by AUFI than females. Males are highly exposed to the environmental pathogens. Male -57%, Female - 43%.

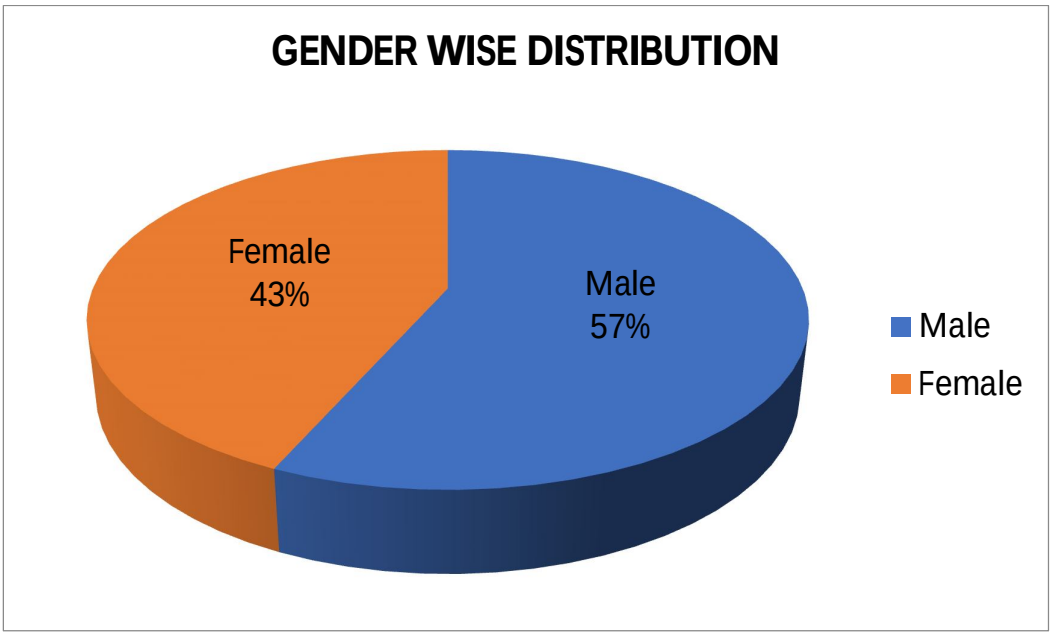


Table-3 : MONTH WISE DISTRIBUTION OF CASES

Month 2016	Blood Culture (5)	PS (9)	Malaria RDT (6)	Dengue		Chikun gunya (IgM) (6)	Lepto (31)	Widal Test (9)	Scrub Typhus IgM (8)
				NS1 (17)	IgM (23)				
April	-	-	-	-		-	-	-	-
May	-	-	-	-		-	-	-	-
June	-	1	-	-		-	-	-	-
July	-	1	-	-		-	-	-	-
Aug	-	-	-	2	2	-	3	-	-
Sep	2	1	1	7	10	1	5	2	2
Oct	2	1	1	8	4	3	8	1	1
Nov	1	1	2	-	2	2	9	3	2
Dec	-	1	-	-	2	-	3	2	2
2017	-	-	-	-	-	-	-	-	-
Jan	-	1	2	3	2	-	3	1	1
Feb	-	1	-	1	1	-	-	-	-
March	-	1	-	-		-	-	-	-
	5	**9**	**6**	**17**	**23**	**6**	**31**	**9**	**8**

AUFI cases more higher in the month of September to December

MONTH WISE DISTRIBUTION OF CASES

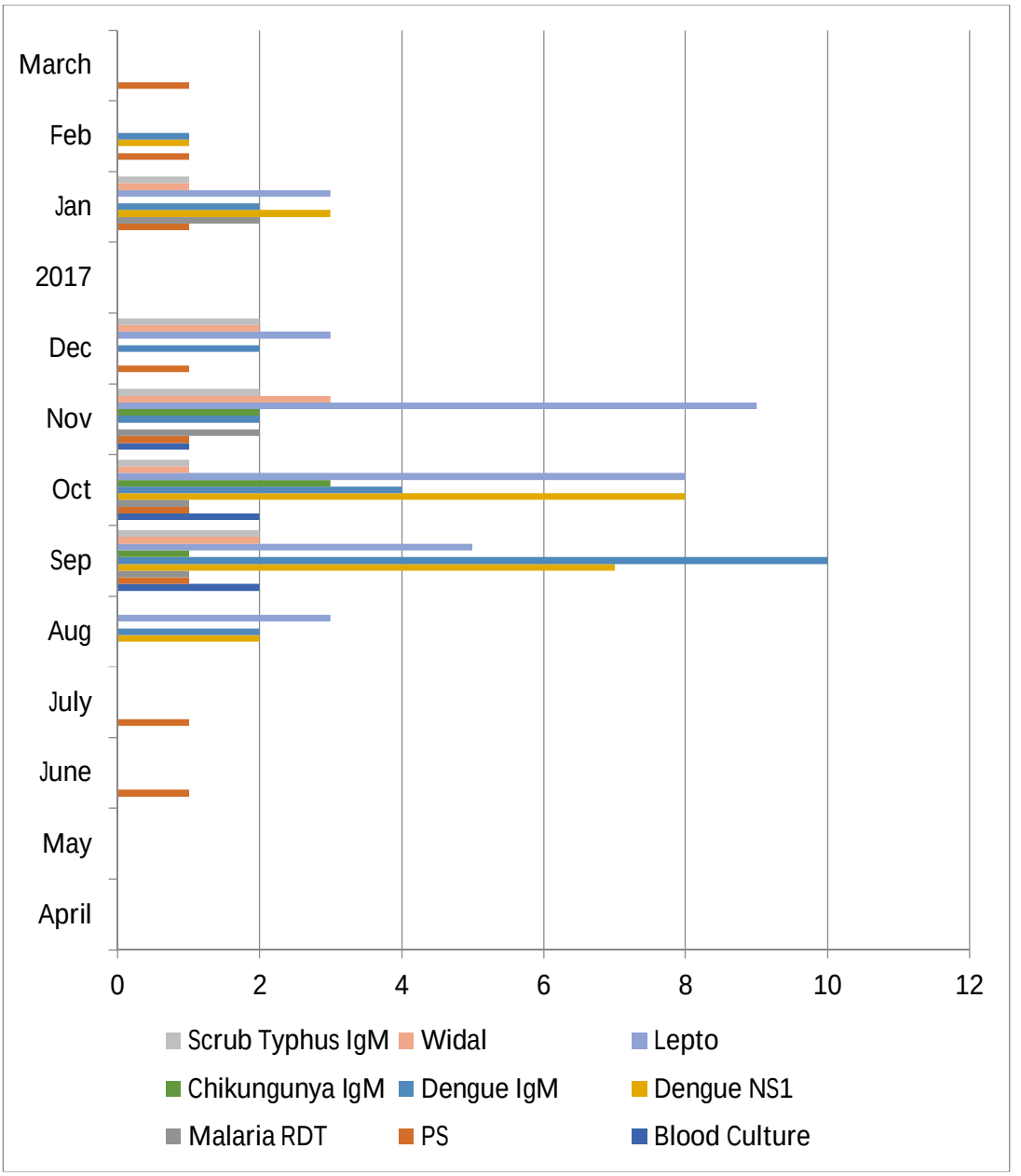


Table-4 : DURATION OF FEVER (n=150)

Days	Number	Percentage
5-10 days	141	94%
11-14 days	9	6%
Total	150	100%

Fever 5-10 days of duration was higher incidence (94%), 10-14 days of fever cases lower (6%).

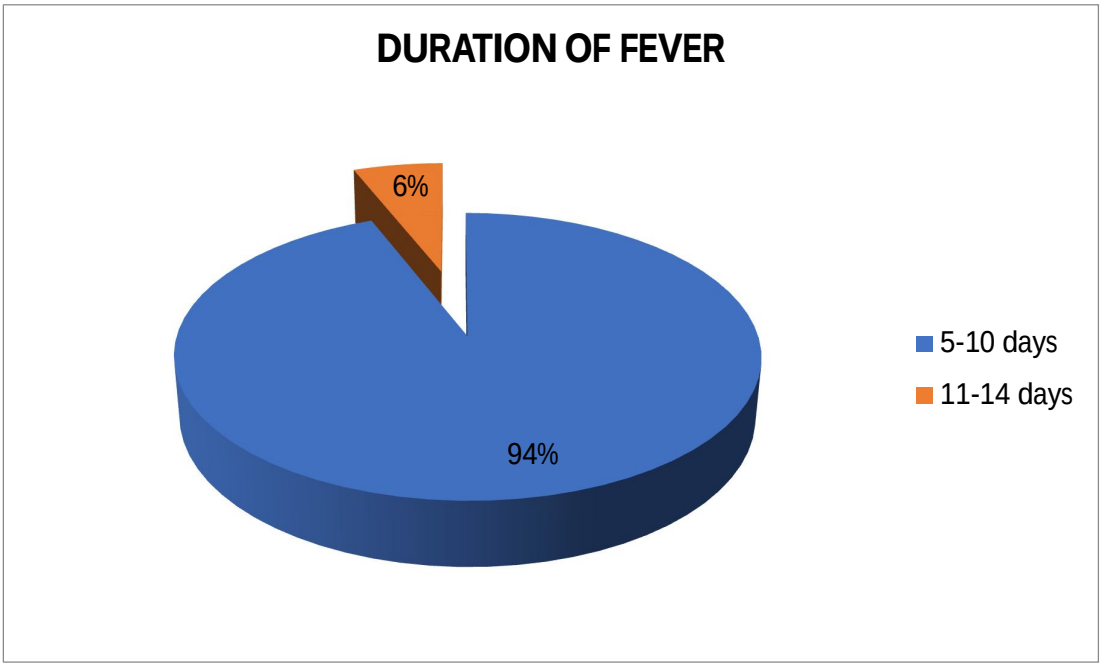


Table-5 : ETIOLOGICAL CLASSIFICATION OF AUFI (n=150)

SI. No	Laboratory Investigations		Number of Positive	Percentage
1	Blood culture		5	3.3%
2	Malaria	Peripheral blood smear	9	6%
		One step malaria Antigen Rapid test	6	
		Both positive (a&b)	6	
3	Dengue by Elisa	NSI Antigen	17	11%
		IgM Elisa	23	15.3%
		Both positive (a&b)	10	
4	Chikungunya		6	4%
5	Macroscopic slide agglutination test for leptospirosis		31	21%
6	Widal test for enteric fever		9	6%
7	Scrub typhus Rapid card test		8	5%
8	Unclassified		42	28%

According to the basis of Etiological classification of AUFI, the 150 samples tested, 40(26.6%) were positive for dengue, 31(21%) for leptospirosis, for malaria 9(6%), for enteric fever 9(6%), for scrub typhus 8(5%), for chikungunya 6(4%), for blood culture for salmonella 5(3.3%), In 42(28%) cases the etiology was unknown. These data are depicted in table.

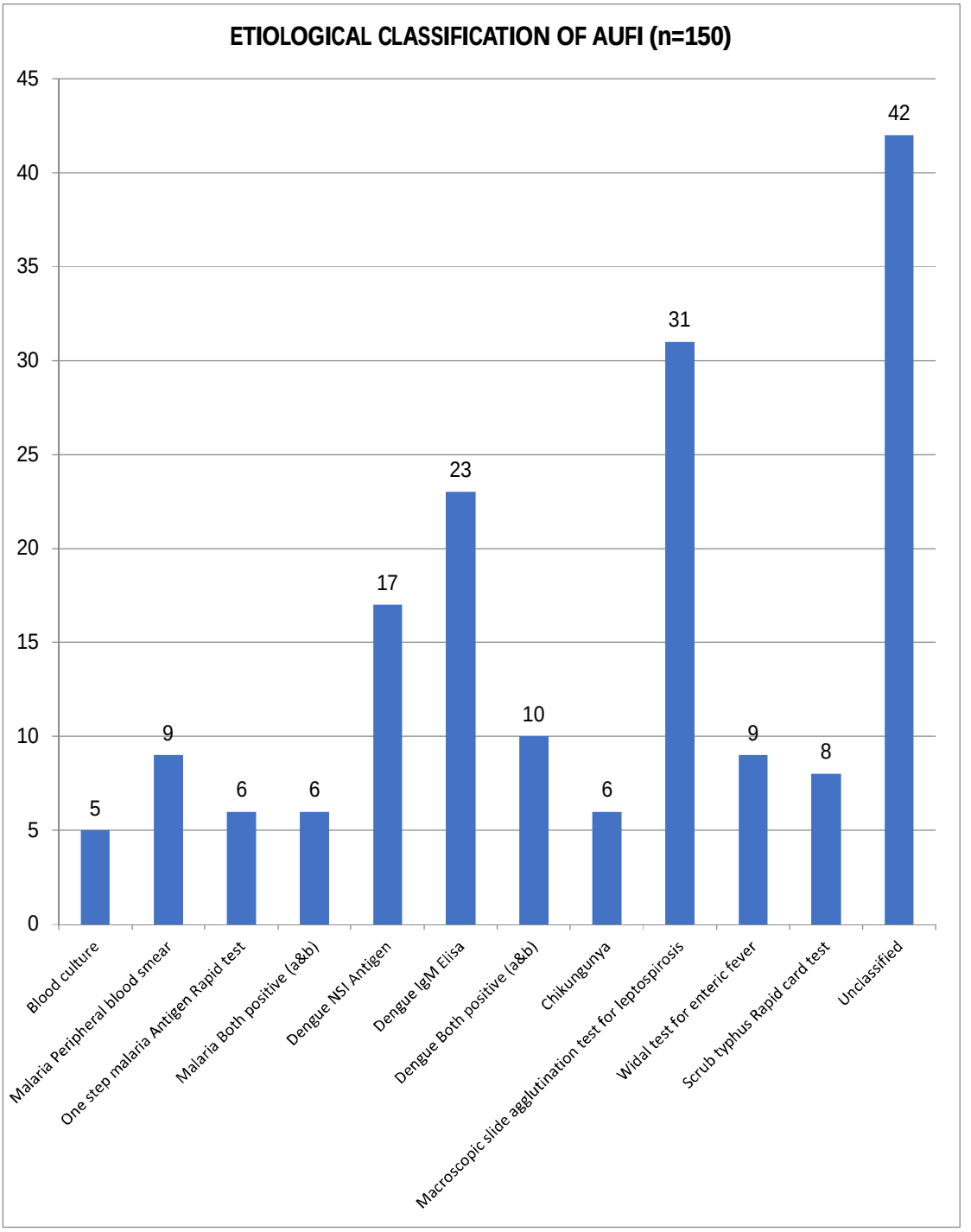

ETIOLOGICAL CLASSIFICATION OF AUFI (n=150)
45
40
35
30
25
20
15
10
5
0
5
9
6
6
17
23
10
6
31
9
8
42
Blood culture
Malaria Peripheral blood smear
One step malaria Antigen Rapid test
Malaria Both positive (a&b)
Dengue NSI Antigen
Dengue IgM Elisa
Dengue Both positive (a&b)
Chikungunya
Macroscopic slide agglutination test for leptospirosis
Widal test for enteric fever
Scrub typhus Rapid card test
Unclassified

Table-6 : LABORATORY PARAMETERS

	Malaria	**Dengue**	**Chikun gunya**	**Leptos pirosis**	**Typhoid**	**Scrub Typhus**
Anaemia	9	-	-	-	-	-
Leucopenia	-	18	6	-	9	
Leucocytosis	-	-	-	-	-	-
Thromobocytopenia	3	32	-	-	-	-
Bilirubin	-	2	-	27	-	-
Urine albumin	-	-	-	5	-	-
SGOT	-	-	-	13	-	-
SGPT	-	-	-	26	-	-

150 Samples were tested. Anemia 9(6%), Leucopenia 18(12%) Thrombocytopenia 32(21.3%), Bilirubin 29(19.3%), Urine albumin 5(3.3%), SGOT 13(8.6%), SGPT 26(17.3%).

Table-7 : CLINICAL FEATURES

Clinical Features	Number	Percentage
Fever with Rigor & Chills	126	84%
Headache	44	29%
Nausea/vomiting	80	53%
Joint Pain	6	4%
Cough/Dyspnea	13	9%
Diarrhea/Loose stools	21	14%
Calf mules tenderness	11	7%
Eschar	6	4%
Rash	7	5%
Arthalgia	7	5%
Altered sensorium	6	4%
Abdominal Pain	43	29%
Bleeding Manifestation	18	12%
Hematamesis	23	15%
Retro orbital pain	6	4%

84% of the patients presented with complaints of fever with rigor and chills followed with 53% with nausea and vomiting 29% with headache and 29% with abdominal pain.

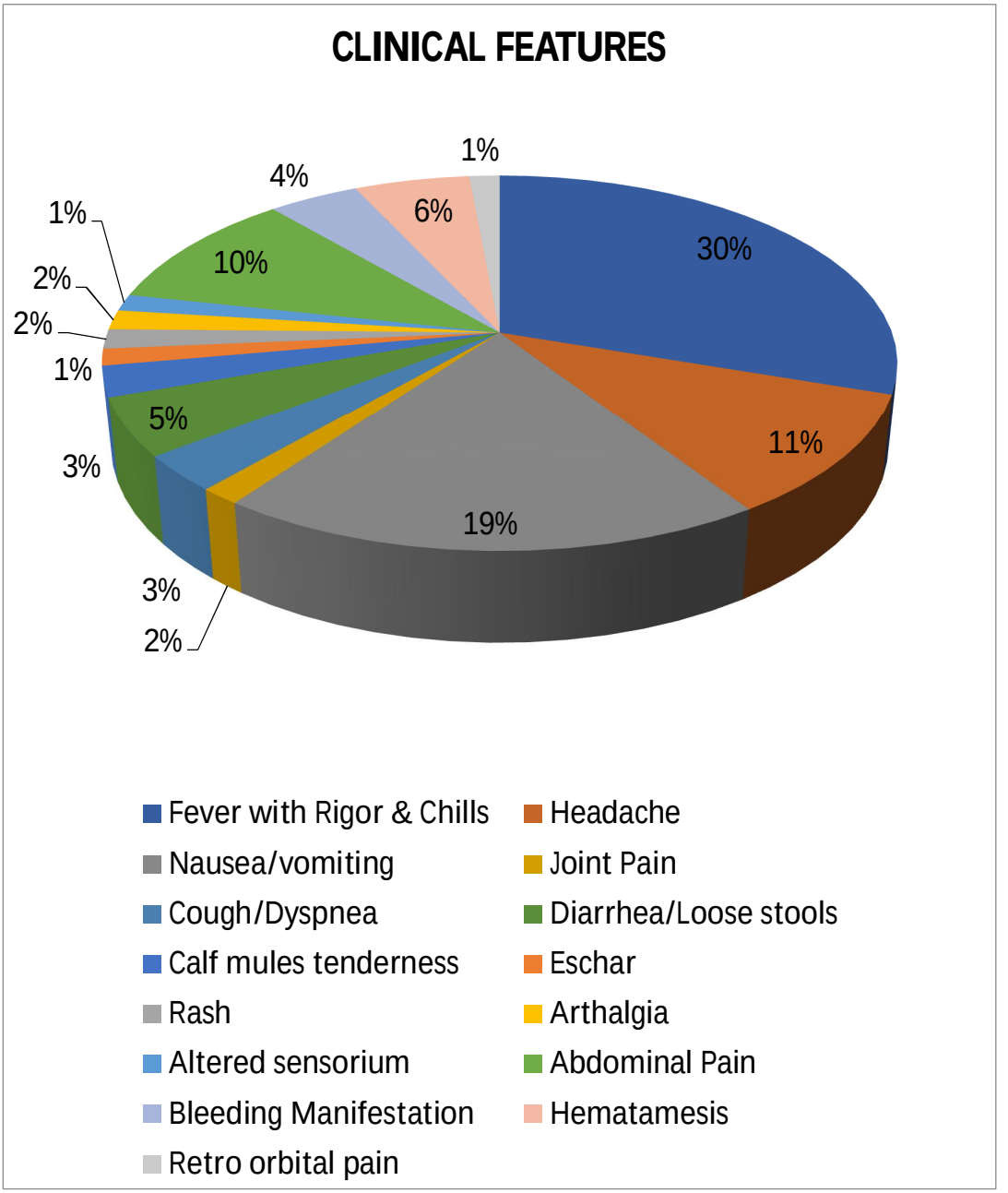

CLINICAL FEATURES
30%
11%
19%
3%
2%
3%
5%
1%
2%
2%
1%
10%
4%
6%
1%
Fever with Rigor & Chills
Headache
Nausea/vomiting
Joint Pain
Cough/Dyspnea
Diarrhea/Loose stools
Calf mules tenderness
Eschar
Rash
Arthalgia
Altered sensorium
Abdominal Pain
Bleeding Manifestation
Hematamesis
Retro orbital pain

Table-8 : AGE WISE DISTRIBUTION OF CAUSES OF AUFI

Total Number of Cases – 150

Total Number of Positive Cases (n=108)

AGE (Years)	Malaria	Dengue NS1 An	Dengue IgM Elisa	Chikun gunya (6)	Lepto (31)	Widal (9)	Scrub Typhus (8)	Blood Culture (5)
18-30	4	10	15	5	15	4	7	2
31-40	2	3	6	-	8	2	-	-
41-50	1	1	2	1	-	2	-	2
51-60	-	-	-	-	-	-	-	-
61-70	2	3	-	-	1	1	-	1
71-80	-	-	-	-	-	-	1	-
	9	17	23	6	31	9	8	5

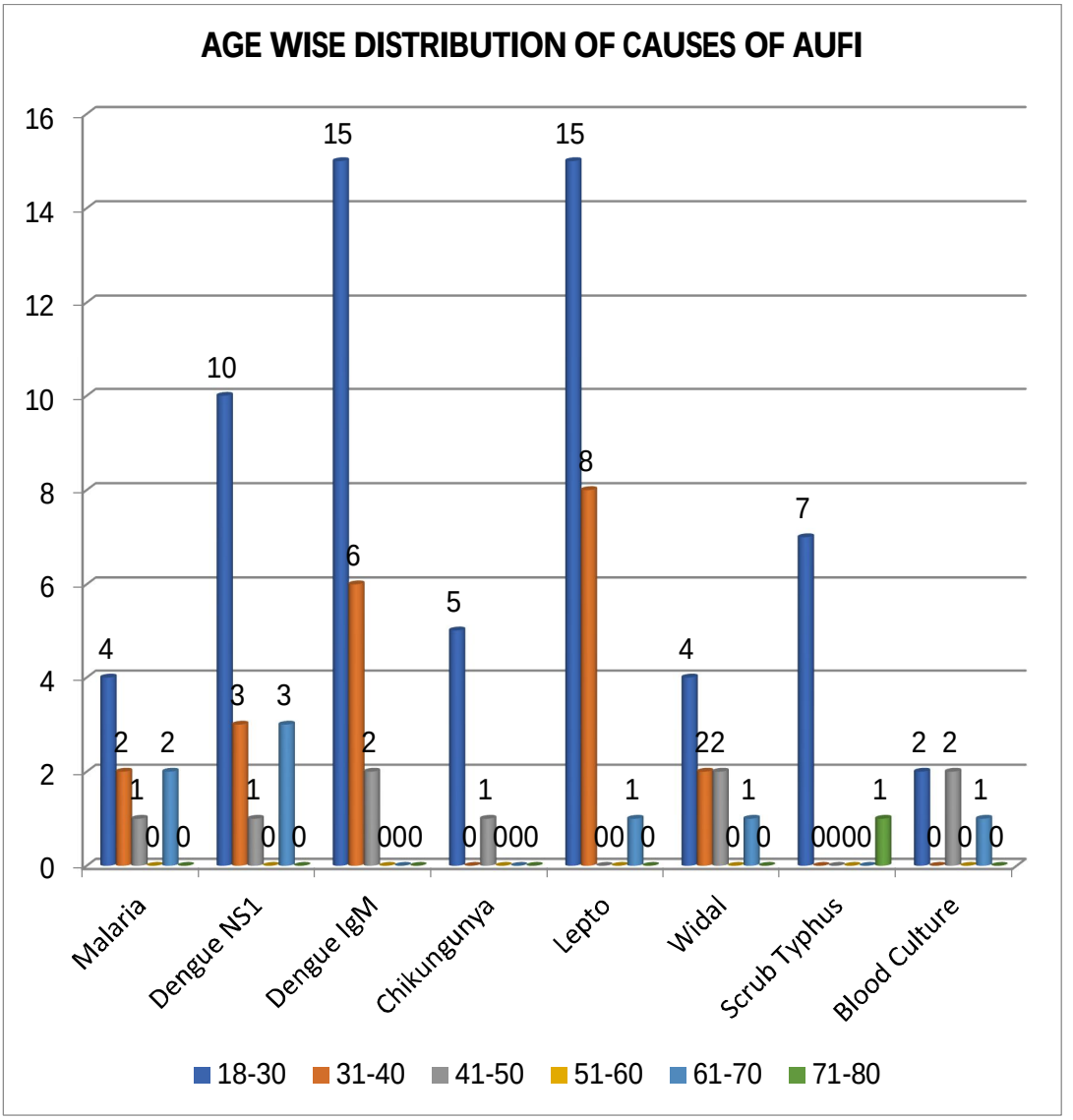

AGE WISE DISTRIBUTION OF CAUSES OF AUFI
16
14
12
10
8
6
4
2
0
Malaria
Dengue NS1
Dengue IgM
Chikungunya
Lepto
Widal
Scrub Typhus
Blood Culture
18-30
31-40
41-50
51-60
61-70
71-80

Table-9 : CLINICAL MANIFESTATIONS OF DENGUE

Fever	Rashes	Vomiting	Headache	Bleeding Manifestation
40	11	14	34	10
100%	28%	35%	85%	25%

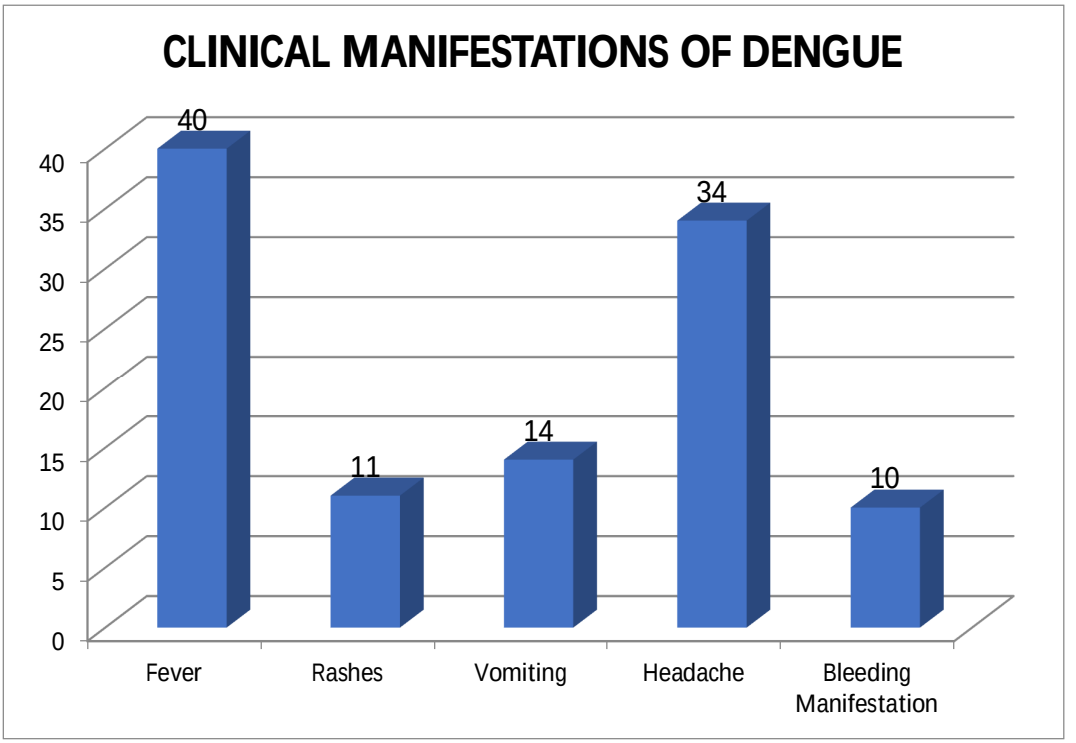


Table-10 : DEFECTION OF MALARIA AMONG AUFI PATIENTS (n=9)

Tests	Number of positives	Percentage
Peripheral blood smear	9	6%
One Step Malaria Antigen rapid test	6	
Both positive a&b	6	

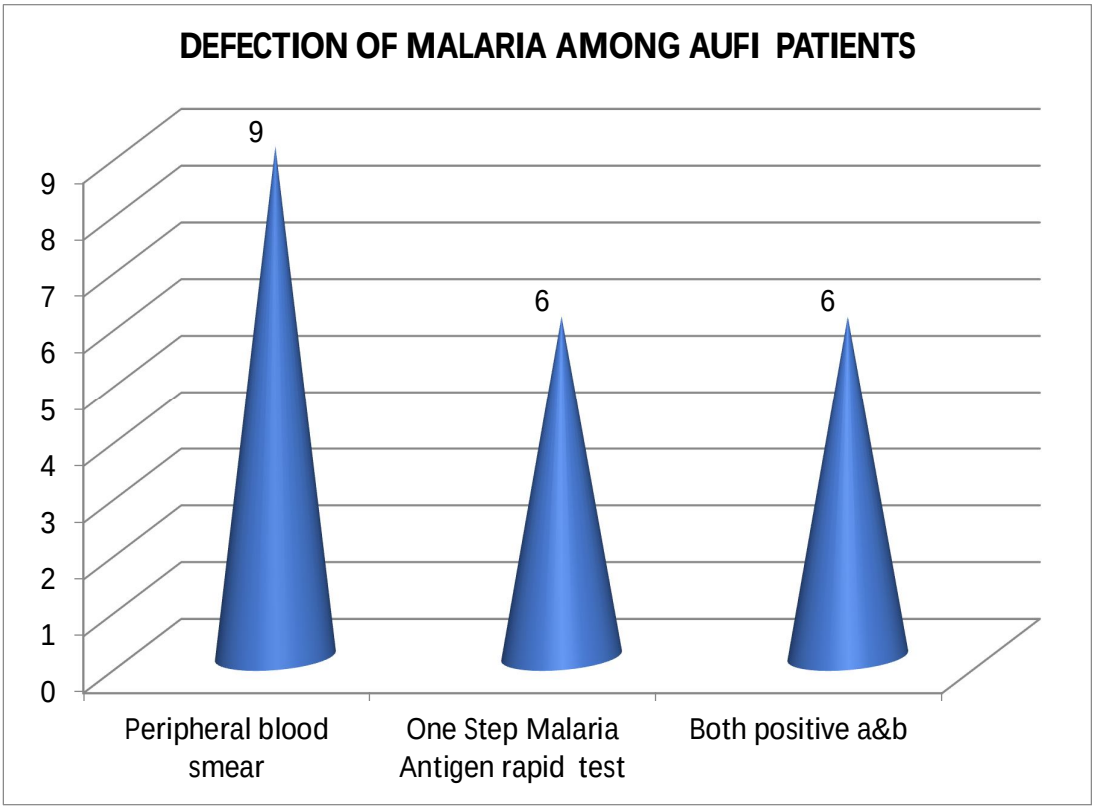
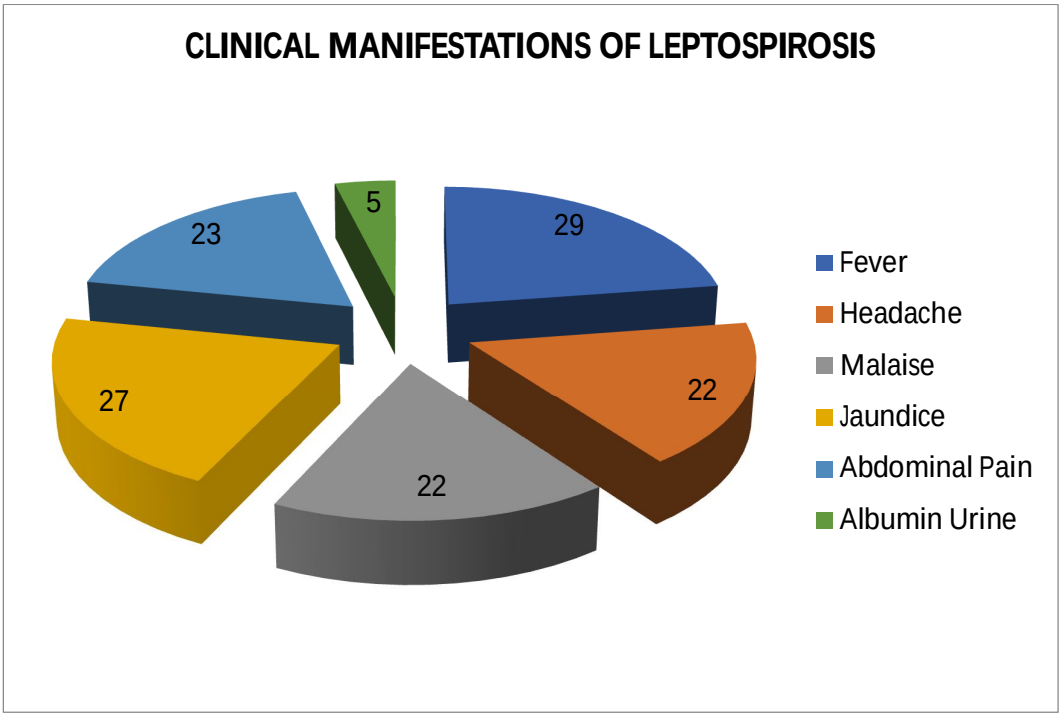


Table-11 : CLINICAL MANIFESTATIONS OF LEPTOSPIROSIS

Total Number of Cases – 150

Total Number of Positive Cases (n=31)

S. No	Presenting Complaints	Numbers	Percentage
1	Fever	29	19%
2	Headache	22	15%
3	Malaise	22	15%
4	Jaundice	27	18%
5	Abdominal Pain	23	15%
6	Albumin Urine	5	3.3%



Discussion

DISCUSSION

Fever is the main clinical symptom of various tropical infectious diseases. The etiologies of human febrile illness can vary region wise in India suggesting that diagnosis, treatment and control programs need to be based on a methodical evaluation of area specific etiologies. Common causes of undifferentiated febrile illnesses include dengue, malaria, leptospirosis, enteric fever, chikungunya and rickettsiae. Reliable laboratory-confirmed diagnosis of AFI require a positive bacteriological \ serological test such as culture results and serological confirmation & pathogen specific antibodies (Immunoglobulin IgM) (or) a four-fold rise in IgG.

This prospective sectional study was conducted in the Institute of Microbiology, Institute of Internal medicine. Rajiv Gandhi Government General Hospital, Chennai during the period of September 2016 - August 2017. The present study includes 150 patients presented with AUFI.

In the present study, among 150 patients with acute febrile illness 85 were male (57%) and 65 were female (43%). And most commonly affected age group were from 18-30 yrs (54.7%) followed by 31-40 yrs(22%). This

was similar to study conducted by Das et al 2015 where male (56%) and female (43%) and commonly affected age group was 18-30 yrs (58.7%).

In this study many cases were reported in September and October which is the rainy and pre monsoon period. This is similar to studies done by Jhansi Charles et al (2015) and Priyadarshini Shanmugam et al. (2016) which showed had peak incidence of cases during rainy and pre monsoon seasons. Since large number of cases occur during rainy and monsoon period, preventive measures have to initiated promptly like control of mosquitoes and rodents. The general public have to be educated and awareness should be created on good hygienic practices, vector control measures and protective measures.

In this study among 150 patients, the duration of fever range was 5-10 days in 94% followed by 11-14 days in 6%. This was similar to study done by Das et al 2015, in which mean duration of fever was 5-7 days (92%).

In this study majority of the cases 126(84%) presented with fever with rigor & chills followed by nausea & vomiting 80 (53%), Headache 43 (29%), abdominal pain (43%). This was in contrast to study Das et al 2015 in which myalgia 113(86%) were more common followed by headache 84(68%).

In this study blood samples from150 patients were subjected to blood culture/serological tested according to the duration of fever. 5(3%) positive for enteric fever by blood culture, 9(6%) positive by widal test for enteric fever. 40(26.6%) were positive for Dengue, 31(21%) were positive for leptospirosis, 9(6%) were positive for malaria 9(6%) were positive for Enteric fever, 8(5%) were positive for scrub typhus, 6(4%) were positive for chikungunya. 31(21%) were unclassified because the etiology was not diagnosed based on these serological tests.

In this study Dengue infections among AUFI, patients were detected by NSI antigen in 17(11.3%) and IgM ELISA in 23(15%) and both were positive in 10 cases (6.6%). This was similar to study done by Vidhyarani 2016, were more marked in Dengue cases with 40 cases of thrombocytopenia and 34 cases of leucopenia. In this study, 40(26.6%) were positive for Dengue. Fever was the commonest symptom for dengue in this study fever followed by headache (42). In this study thrombocytopenia 32(21.3%) and leucopenia 25(16%). This was similar to the study conducted by Vidhyarani et al 2016, and by Yogesha et al 2014.

In this study detection of leptospirosis by MSAT among 150 patients was 31 (21%). Most common presentation of leptospirosis was fever

29(19%) followed by jaundice 27(18%). This was similar to study done by Neelu sree p. et. al 2015 where the detection rate of leptospirosis was 22%.

In this study detection of malaria among 150 patients were 9(6%). By using peripheral blood smear the detection rate was 100% when compared to one step malaria Antigen rapid test which was 66%. The most common presentation of malaria was anaemia (9) followed by thrombocytopenia (3). This was similar to study done by Kashinkunti et al 2013 were detection rate was 100% in peripheral blood smear compared to rapid test (80%).

In this study scrub typhus and chikungunya were detected in 8(5%) and 6(4%) cases respectively. It was low when compared to the study by Kumar. V. et. al. 2014 and Dipmala Das et al 2015 were the prevalence was 33%. This is due to geographical distribution of the causative agent.

Blood culture was positive in 5 cases for Salmonella typhi infection. and Widal test for salmonella Typhi positive (9) in this study. The similar study was done by Bhan mk, Bahl R, Bhatnagar, Lancet. 2005:366 (9487):749-62.

Summary

SUMMARY

The present study includes 150 patients admitted with Acute undifferentiated febrile illness

- Among 150 patients 85(57%) were male and 65(43%) were females.
- Most commonly affected age group were 18-30yrs (54.7%) followed by 31-40 yrs (22%).
- Majority of cases were reported in September and October(68) followed by November and December (33).
- Maximum duration of fever were 5-10 days (94%) followed by 11-14 days (6%).
- Majority of them presented with fever with rigor & chills 126(84%) followed by nausea and vomiting 80(53%).
- Majority of them were positive for Dengue 40(26.6%) followed by leptospirosis 31(21%), malaria were positive in 9(6%), Enteric fever were positive in 9(6%) by widal test & one step RDT positive 8(5%) for scrub typhus, 6(4%) were positive for chikungunya, 42(28%) of them were unclassified.
- Among 50 patients positive for dengue 17 patients were detected by NSI antigen 33 by Dengue IgM ELISA . Majority of dengue patients

presented with fever (50) followed by headache (42), and showed thrombocytopenia (32) and leucopenia (18).

- Among 150 patients 31(21%) were positive for leptospirosis detected by microscopic standard agglutination test (100%).
- Among 150 patients, 9(6%) were positive for malaria and it was detected by peripheral smear (100%)
- 8(5%) and 6(4%) were positive for scrub typhus and chikungunya which was detected by Rapid card test and IgM ELISA kit respectively.

Conclusion

CONCLUSION

In this study, 150 patients admitted with signs and symptoms of acute undifferentiated febrile illness patients were tested by blood culture and for various serological tests according to the duration of fever. 40(26.6%) were positive for Dengue, 31(21%) were positive for leptospirosis, 9(6%) were positive for malaria, 9(6%) were positive for enteric fever by widal, 8(5%) were positive for scrub typhus, 6(4%) were positive for chikungunya, and 42(28%) were negative for all these tests hence the etiology was not known.

Majority of them showed peak seasonal incidence of cases during rainy and post monsoon seasons, preventive measures have to initiated like control of mosquitoes, rodents and the general public have to be educate and awareness should be created on good hygienic practices, vector control measures and protective measures.

Although Dengue being the commonest cause of acute febrile illness, other different causes prevailing in tropical countries should be suspected and correct diagnosis is crucial to prevent the delay in starting appropriate therapy.

Confirmatory diagnostic tests with good correlation of clinical findings, relevant laboratory parameters and epidemiology of disease is essential to prevent complications and to reduce morbidity and mortality in patients with acute febrile illness.

Antimicrobial susceptibility pattern wass in s.Typhi with re-reemergence of susceptibility of ampicillin chloramphenical, and cotrimoxarole. S.Typhi was high resistance to Nalidixic acid and pefloxacin. The use of pefloxacin as a surrogative marker for fluoroquinole susceptibility is promising, since it detects all the mechanisms for fluoroquinolone resistance in this study, and clearly separates peflax susceptibility and resistant strain.

In this study, unclassified 42 cases (28%) in which causes may be due to Influenza, Hepatitis B, Infective endocarditis, UTI and LRI.

COLOUR PLATES

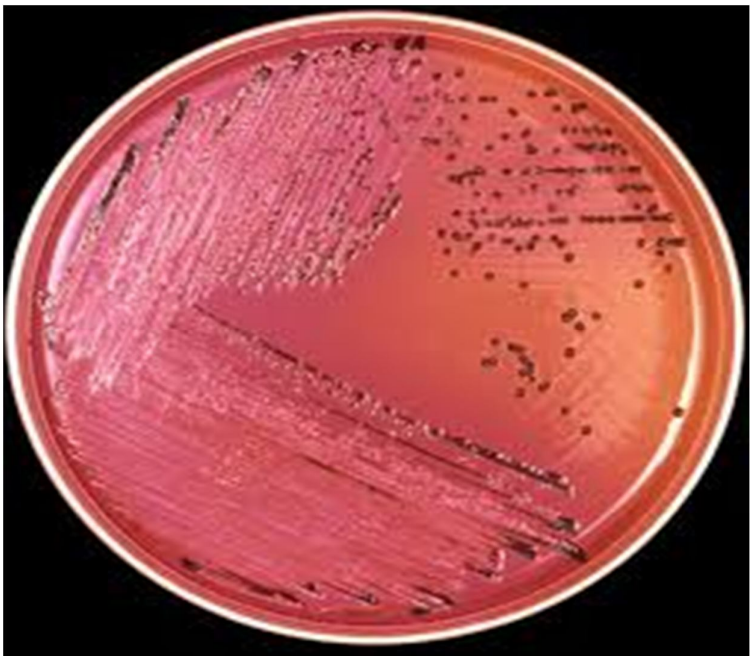


Image 1 : Salmonella Typhi (Red Colour colonies with black center)

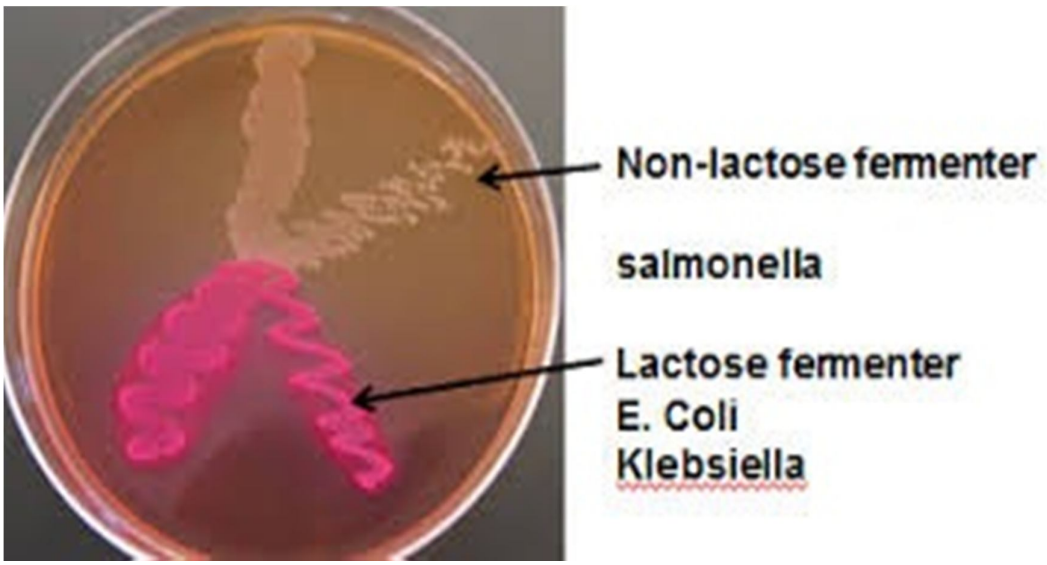


Image 2 : Mac Conkey Agar Plate LF & NLF

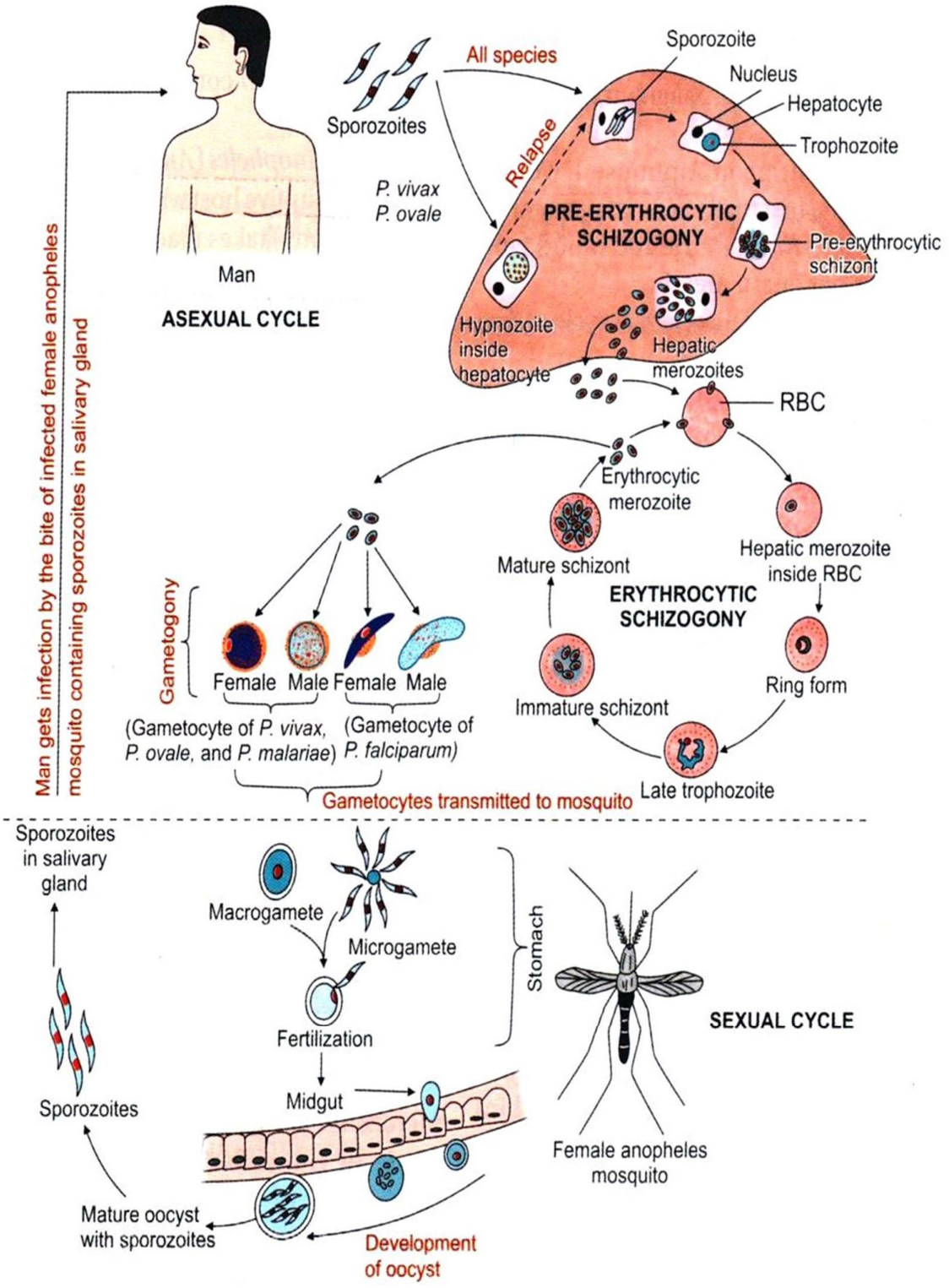


Image 3 : Life cycle of Malaria Parasite

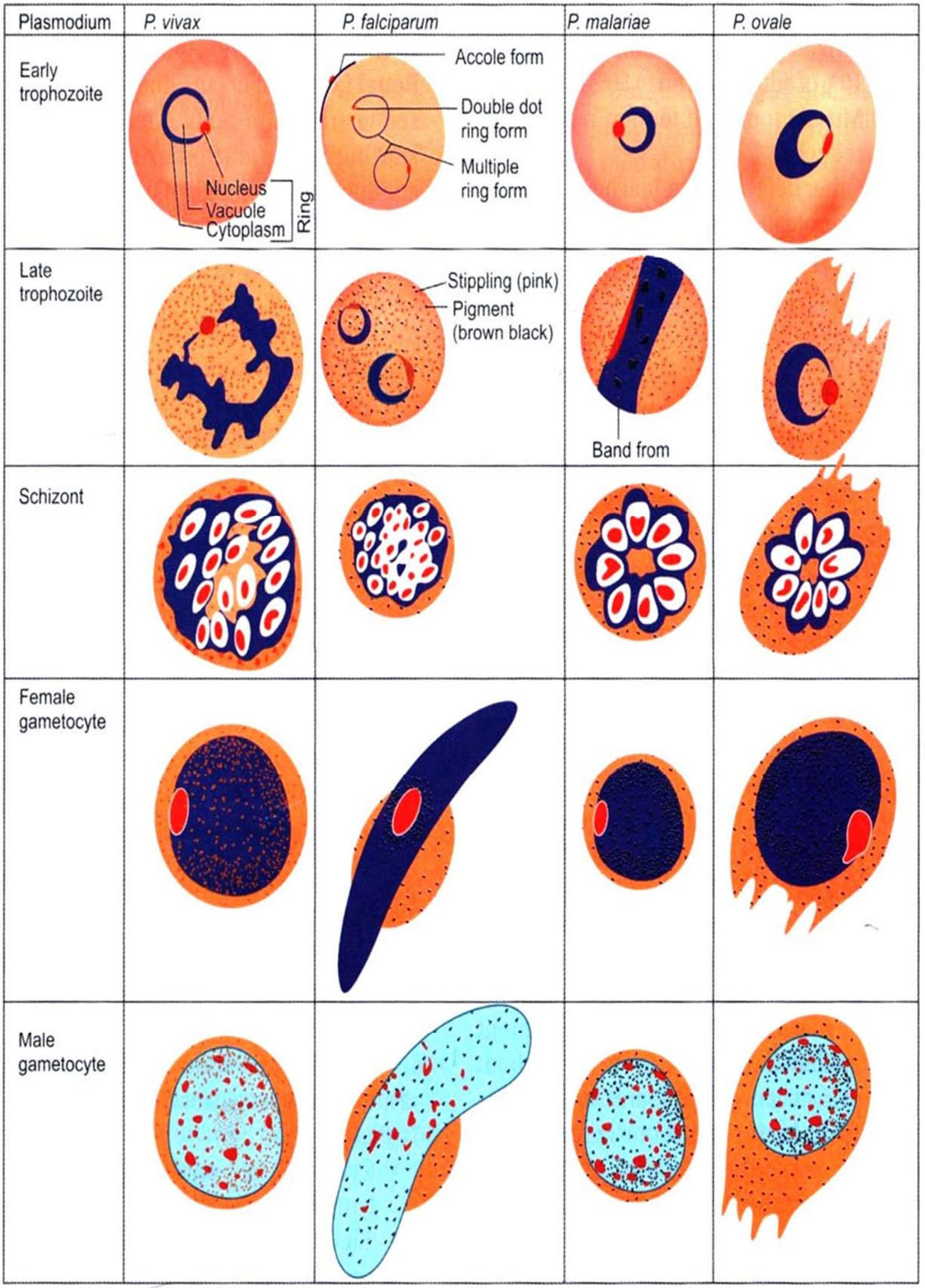


Image 4 : Stages of Plasmodium species

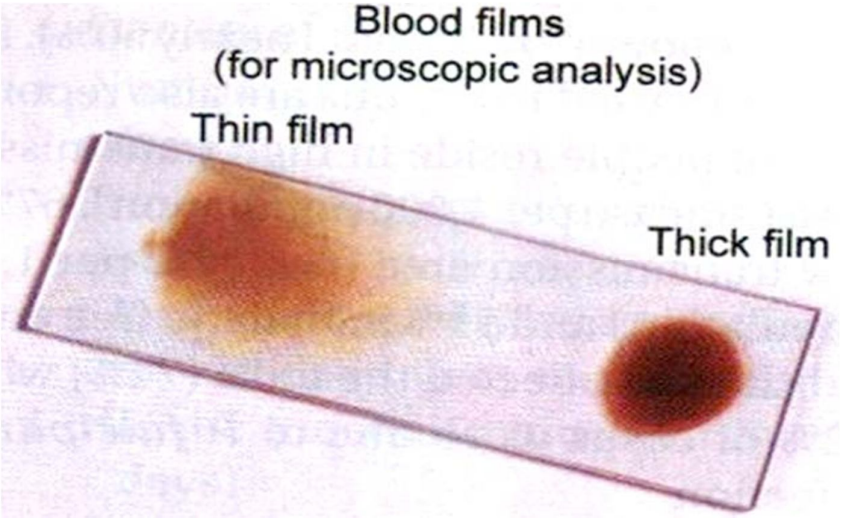


Image 5 : Peripheral Smear for Malaria

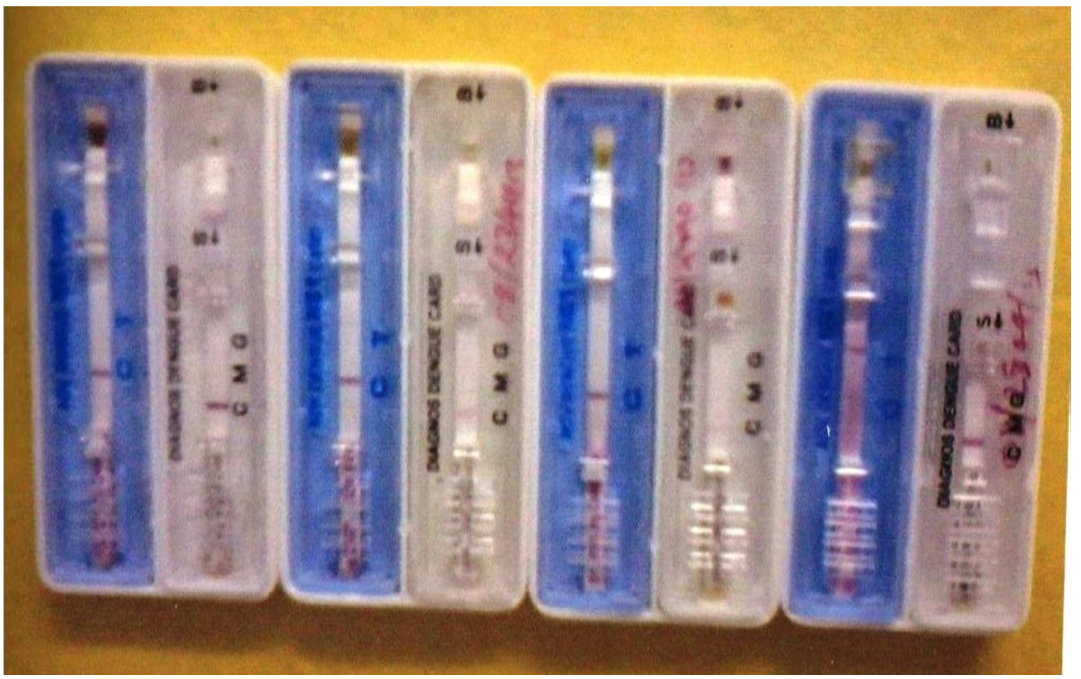


Image 6 : Positive and Negative NSI – Antigen

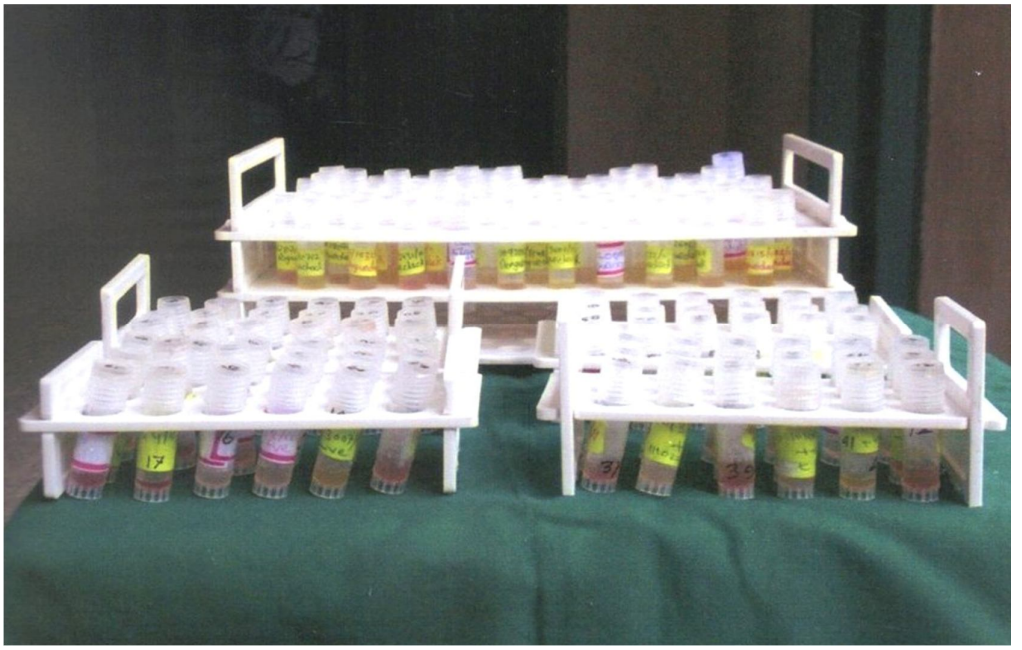


Image 7 : Serum samples taken for Dengue early capture ELISA & ICT Card Test

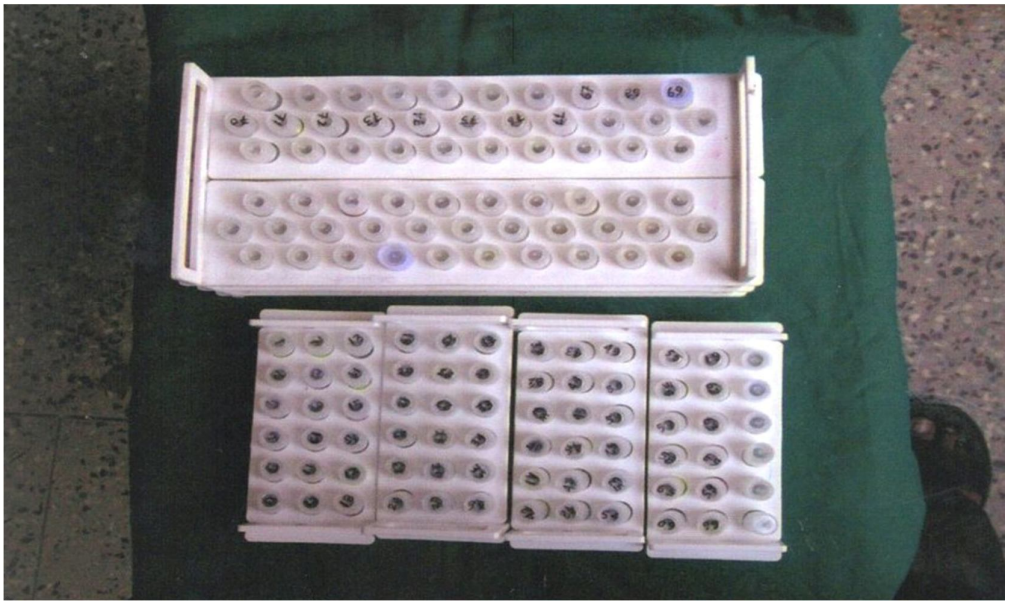


Image 8 : Serum samples taken for Dengue early capture ELISA & ICT Card Test

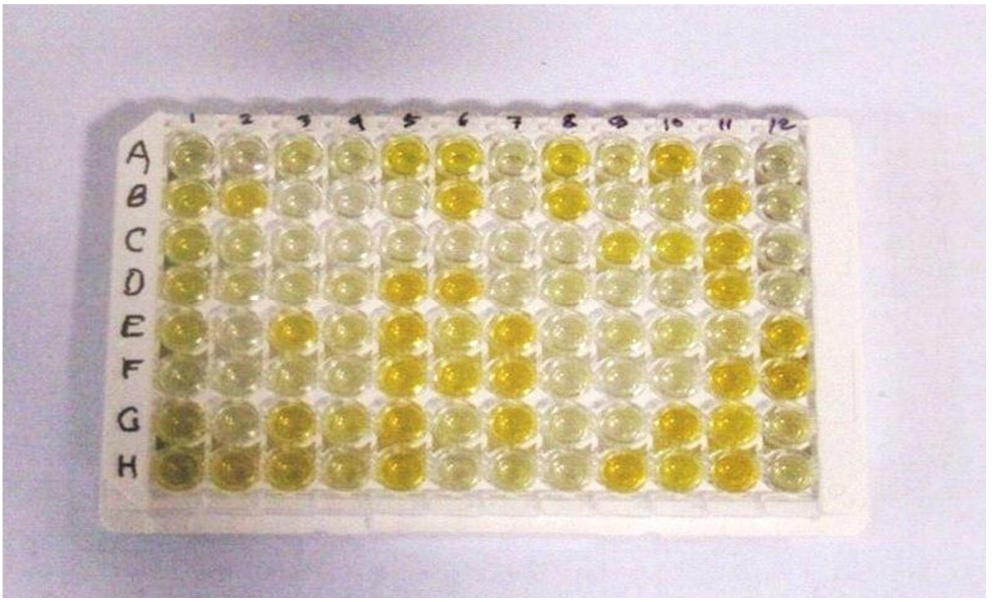


Image 9 : Dengue NS1-Ag. Early capture ELISA microtiter plate shows positive & negative results

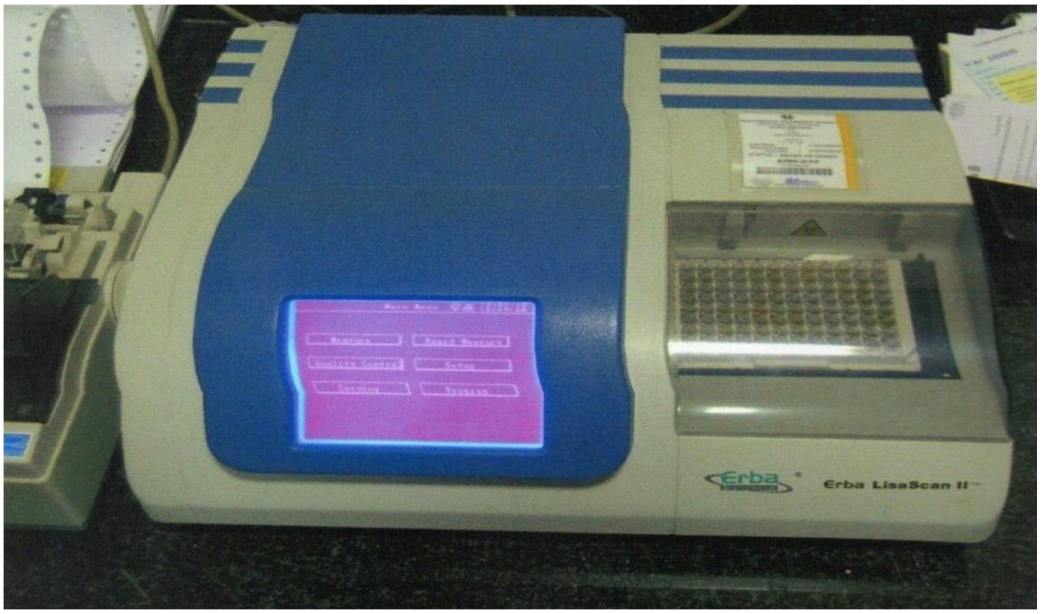


Image 10 : Spectrophotometer (ELISA Reader) with Microtiter plate

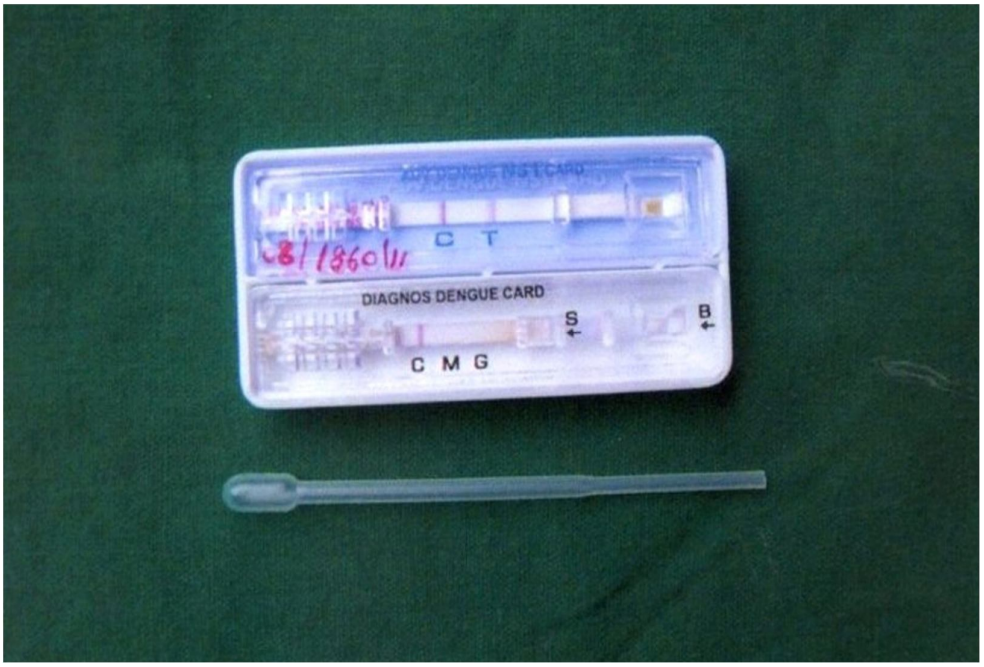


Image 11 : Dengue NSI Antigen Positive Strip

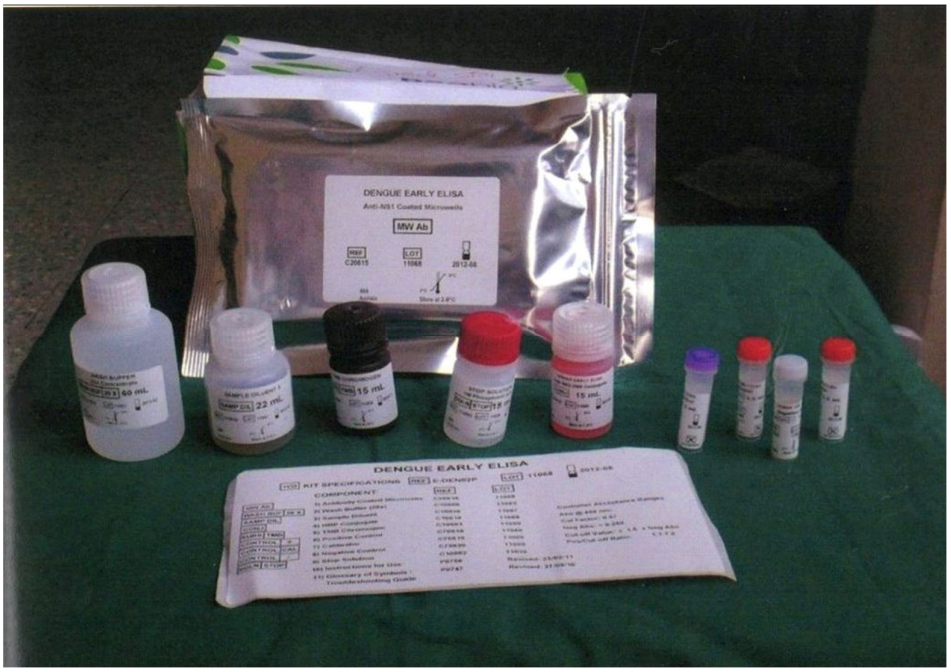


Image 12 : Dengue NS – I Ag. Early Capture ELISA Test Kit (PANBIO)

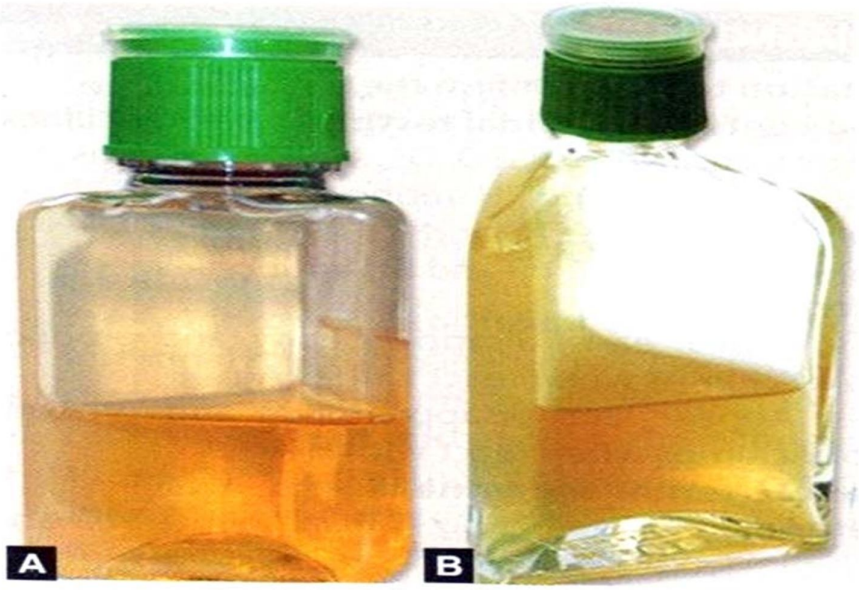


Image 13 : Blood culture bottles A. Monophasic medium (BHI broth) B. Biphasic medium (Castaneda's), containing BHI broth and BHI agar

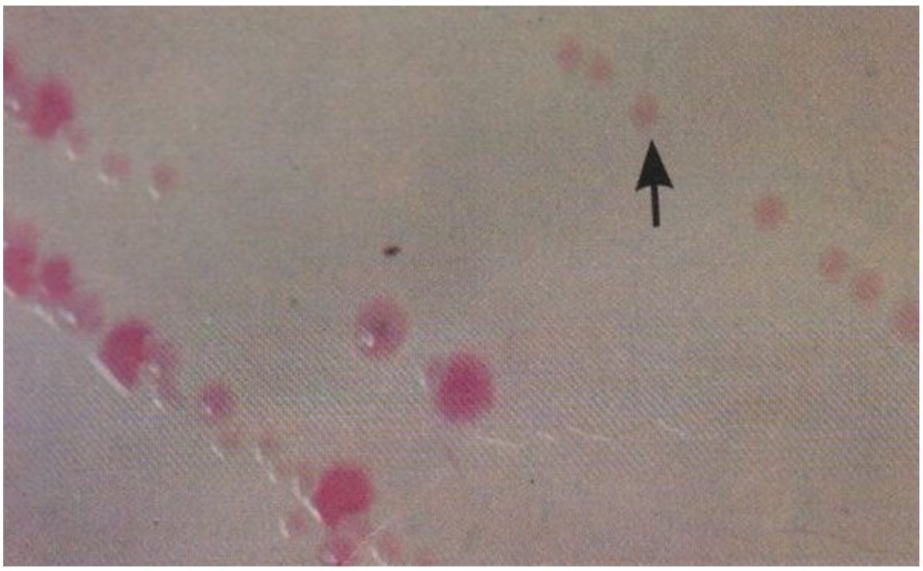


Image 14 : Colourless colonies in Mac Conkey agar (NLF)

Image 15 : ELISA Multiscan system

Image 16 : One Step Malaria Antigen Rapid Test

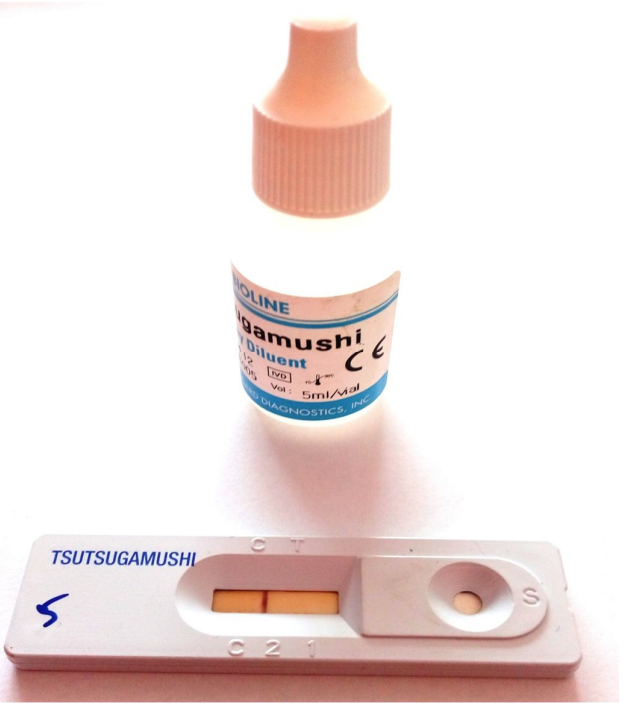


Image 17 : One Step Scrub Typhus antibody test

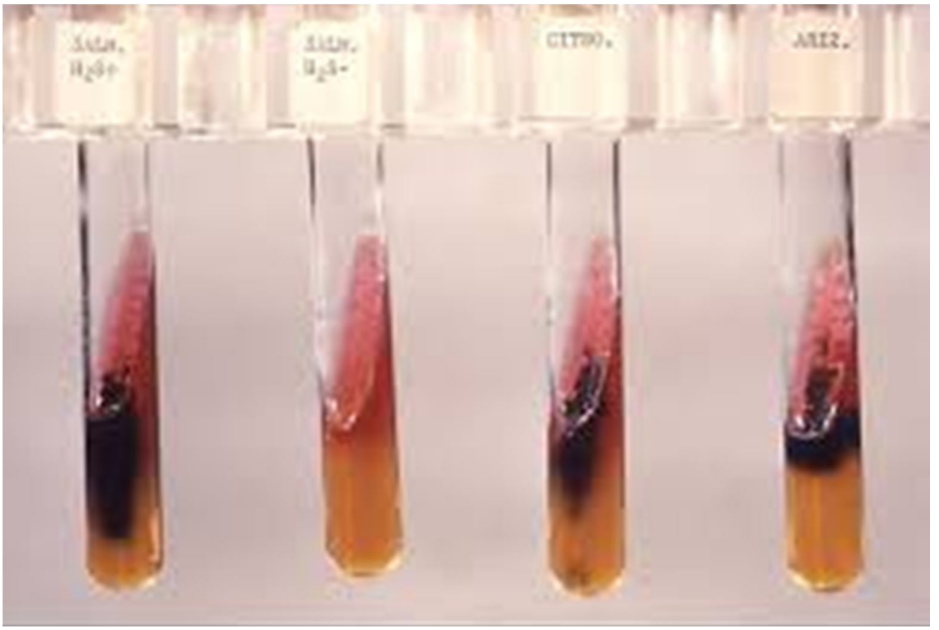


Image 18 : Triple Sugar Iron (TSI) Agar Test

Image 19 : Leptospirosis (MSAT)

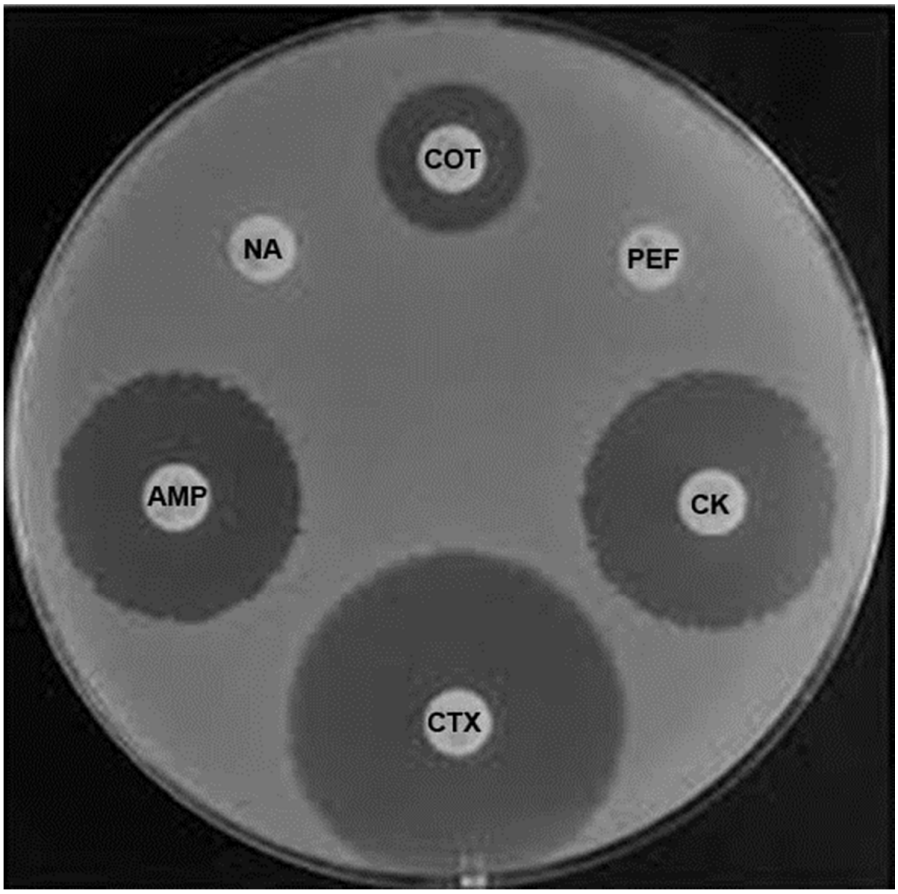


Image 20 : Antibiotic Sensitivity pattern

(Disc Diffusion Method)

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ANNEXURE-I

ABBREVATIONS

AUF	-	Acute undifferentiated Fever
AFI	-	Acute Febrile Illness
UUF	-	Unclassified Undifferentiated Fever
AUFI	-	Acute Undifferentiated Febrile Illness
MAC	-	Mac Conkey Agar Plate
BAP	-	Blood Agar Plate
NA	-	Nutrient Agar Plate
BHI	-	Brain heart Infusion
MH	-	Mueller Hinton Agar Plate
ELISA	-	Enzyme Linked Immuno Absorbant Assay
DV	-	Dengue Virus
DF	-	Dengue Fever
DHF	-	Dengue Haemorrhagic Fever
DSS	-	Dengue shock Syndrome
MSAT	-	Microscope Slide Agglutination Test
NS1	-	Non Structural Protein
OD	-	Optical Density
PH	-	Meassure of acidity/basicity of a solution
WHO	-	World Health Organisation
IgG	-	Immunoglobulin G

IgM	-	Immunoglobulin M
PGE	-	Prostaglandin
ULV	-	Ultra Low Volume
ICUT test	-	Indole, citrate, urease, Triple sugar iron test.
H2S	-	hydrogen sulfide

Antibiotics

Amp	-	Ampicillin
Cotri	-	cotrimoxazole
Nal	-	Nalidixic acid
Ck	-	Chloramphenical
Cef	-	cefriaxone
Pef	-	pefloxacin

APPENDIX – II

A. Dengue NS1 Antigen

Principle of the test

This is a solid phase enzyme linked immunosorbent assay (ELISA) based on the "Direct Sandwich" principle. The microwells are coated with Anti-dengue NS1 antibodies with high reactivity for Dengue NS Ag. The samples are added in the wells followed by addition of enzyme conjugate (monoclonal anti-dengue NS1 antibodies linked to Horseradish Peroxidase (HRPO)). A sandwich complex is formed in the well wherein dengue NS1 (from serum sample) is "trapped" or "sandwiched" between the antibody and antibody HRPO conjugate. Unbound conjugate is then washed off with wash buffer. The amount of bound Peroxidase is proportional to the concentration dengue NS1 antigen present in the sample. Upon addition of the substrate buffer and chromogen, a blue colour develops. The intensity of developed blue colour is proportional to the concentration of dengue NS1 antigen is sample. To limit the enzyme-substrate reaction, stop solution is added and a yellow colour develops which is finally read at 450nm spectrophotometrically.

Kit & Its components

Microwells	Microwells coated with anti-Dengue NS1 antibodies packed in a sealed pouch with desiccant
Diluent	Buffer containing protein stabilizers & antimicrobial agents as preservative and to be used for Sample & Conjugate dilution.
Enzyme Conjugate Concentrate (50X)	Containing Monoclonal Anti-Dengue NS1 linked to horseradish Peroxidase with protein stabilizers.
Wash Buffer Concentrate (25X)	Concentrated phosphate buffer with surfactant.
TMB Substrate	TMB, to be diluted with TMB Diluent before use.
TMB Diluent	Buffer solution containing H2O2
Control(-)	Normal human serum negative for Dengue NS1 antigen with preservative.
Control(+)	Recombinant Dengue NS1 antigen with Preservative
Calibrator	Recombinant Dengue NS1 antigen with Preservative
Stop solution	Ready to use, 1N H2SO4
Plate sealers	Adhesive sheets to cover the microwells during incubation.

B. Dengue IgM Capture ELISA

Principle of the test

Serum antibodies of the IgM class, when present, combine with anti-human IgM antibodies attached to the polystyrene surface of the microwell test strips. A concentrated pool of Dengue 1-4 Antigens is diluted to the correct working volume with Antigen Diluent. The antigens are produced using an insect cell expression system and immunopurified utilizing a specific monoclonal antibody. An equal volume of the HRP conjugated Monoclonal Antibody (MAb) is added to the diluted antigen, which allows the formation of antigen-MAb complexes. Residual serum is removed from the assay plate by washing, and complexed antigen-MAb is added to the assay plate. After incubation, the microwells are washed and a colourless substrate system, tetramethylbenzidine/hydrogen peroxide (TMB chromogen) is added. The substrate is hydrolysed by the enzyme and the chromogen changes to a blue colour. After stopping the reaction with acid, the T<B becomes yellow. Colour development is indicative of the presence of anti-dengue IgM antibodies in the test sample.

Components
Wash Buffer 20X Concentrate
TMB Chromogen
Stop solution
Sample Diluent
Antigen Diluent
Dengue 1-4 Antigen
Dengue IgM Capture MAb Tracer HRP
Anti-Human IgM Antibody coated Microwells
Dengue IgM capture positive control
Dengue IgM capture calibrator
Dengue IgM capture Negative control

C. CHIKUNGUNYA IgM CAPTURE ELISA KIT

Principle

IgM antibodies in the patient's serum are captured by anti-human IgM (µ chain specific) coated on to the solid surface (wells). In the next step, CHIK antigen is added which binds to captured human IgM in the sample. Unbound antigen is removed during the washing step. In the subsequent step biotinylated anti CHIK monoclonal antibodies are added followed by Avidin-HRP. Subsequently, chromogenic substrate (TMB/H2O2) is added, the reaction stopped by 1N H2SO4. The intensity of color/optical density is measured at 450nm.

COMPONENTS

a) Anti Human IgM coated wells(96) Ready to use

(Twelve strips with eight wells each)
Ninety six polystyrene wells are coated with polyclonal rabit anti Human IgM and post coated to block non-specific binding of any protein; and also to stabilize the coated antibody. Stable at 2-8°c if protected from

b) Sample Diluent for Chik IgM Ready to use
One bottle (60ml)/kit.
Phosphate Buffered Saline with additives to be used for dilution of test samples (serum).

c) Wash Buffer Concentrate (20X)
One bottle (60ml)/kit.
Phosphate buffered saline with surfactant and antibioticsw. If the Wash Buffer Concentrate shows crystallization, warm the bottle at 37°c until crystallization disappears. Before use, dilute wash buffer concentrate 1:19 (1 part of buffer concentrate + 19 parts of high grade distilled water). For essay of eight samples (six clinical samples and two controls), 100ml of diluted wash buffer is sufficient.

d) Chikungunya Antigen Ready to use
One vial (6ml)/kit
CHIK antigen in stabilizer with additives. The antigen may show pink/yellowish color due to the phenol red indicator. Unused antigen must be decontaminated before it is discarded.

e) Anti CHIK monoclonal antibody (Biotin labeled) Ready to use
One vial (6ml)/kit.
Anti CHIK Monoclonal Antibody-Biotin labeled diluted in stabilizer with additives and antibiotics.

f) Avidin-HRP-Ready to use
One vial (6ml)/kit.
Avidin-HRP diluted in stabilizer with additives and antibiotics. Protect the solution from direct exposure to light.

g) Liquid TMB substrate-Ready to use
One vial (12ml)/kit.
Tetramethylbenzidine Dihydrochloride. It is light sensitive. Protect from light.

h) Stop solution Ready to use
One vial (12ml)/kit
1 N H_2SO_4 wear protective gloves mask and eye glasses while handling stop solution.

i) CHIK IgM Positive control Ready to use
One vial (0.8ml)/kit
Human serum positive for CHIK IgM antibodies diluted in stabili8zer with Additives. This is ready to use reagent. Do not dilute.

j) CHIK IgM Negative Control Ready to use
One vial (0.8ml)/kit
Human serum Negative for CHIK IgM antibodies diluted in stabili8zer with Additives. This is ready to use reagent. Do not dilute.

APPENDIX – III

Preparations:

A. Mac Conkey Agar

Uses: Cultivation and differentiation of Entro bacteria.

Ingredients:

Peptone	-	20gm/lit
Lactose	-	10gm
Neutral red	-	0.04
Water	-	1 lit
Sodium Taurochlotate	-	5gm
Agar	-	5.0gm
PH	-	7.4 ± 0.2

Orange red, clear to slightly opalescent gel.

Directions:

1. suspend 55.08ms of the powder in 1000ml of distilled water.
2. Mix thoroughly
3. Boil with frequent agitation to dissolve the powder completely. U.
4. Sterilize by autoclaving at 121°c (15 1bs pressure) for 15 minutes.

Quality control – E.coli (positive) Shigalla flexneri.(Negative)

B. Blood Agar

It is enriched medium and indicator medium showing the haemolytic properties of bacteria.

The medium is prepared by adding sterile blood to sterile nutrient agar that has been melted and cooled to 50°c.

It consist of 10% sheep blood agar, double layered, and translucent.

A thin layer of melted nutrient agar about 7ml for a 90cm petri dish, is poured and allowed to set. Then a similar thin layer of 10% sheep blood agar is poured on top of the first layer.

C. Nutrient Agar

Uses: For cultivation of less fastidious microorganisms can be enriched with blood and other body fluids.

Ingredients:

Peptone	-	5gms/lit
Nacl	-	5gms/lit
Meat extract (Beaf)	-	1.50gms/lit
Yeast Extract	-	1.50gms/lit
Agar	-	15gms/lit
Water	-	1 lit

Directions:
Suspend 28.0gms in 1000mlof distilled water, Heat to boiling to dissolve the medium completely.
Sterlize by autoclaving at 15Ibs pressure 121°c for 15 minutes. Cool to 45-50°c. Mix well and pour into the petri dish.

D. Muller Hinton Agar
Uses: For determination of susceptibility of micro organisms to Antibacterial agents.

Ingredients:

Meet infusion from	-	300.00 gms/lit
Casein acid hydrolysate	-	17.50 gms/lit
Starch	-	1.50 gms/lit
Agar	-	17.00 gms/lit
Water	-	1 lit
PH	-	7.3 ± 0.1

Directions:
Suspend 38.0 gms in 1000ml of distilled water. Heat boiling to dissolve the medium completely. Sterilize by Autoclaving at 15 Ibs pressure, 121°c for 15 minutes and cool to 45-50°c mix well.

E. Preparation of Leishman's Stain:
The stain is available in the form of powder or tablet. The strength of the solution used for staining is 0.15 per cent of the stain in methyl alcohol (0.15 gram of the stain is dissolved in 100 ml of acetone-free pure methyl alcohol). The materials required for preparation are a ground-glass stoppered bottle of 150ml capacity, a 100 ml graduated glass cylinder and a glass pestle and mortar. All the articles are thoroughly cleaned and before use, are rinsed with methyl alcohol. The amount of methyl alcohol (100 ml) is first measured in the graduated cylinder. The weighed amount of Leishman's powder (0.15 g) or the requisite number of tablets (each tablet contains 0.15 g of the stain) is placed in the glass mortal and is ground into a paste by adding methyl alcohol in small quantities (about 2 ml each time). The dissolved stain is carefully decanted off, from time to time, into the glass-stoppered bottle. The undissolved stain is ground again with methyl alcohol till no residue is left and the whole methyl alcohol has been used up. The stoppered glass-bottle with the stain is kept in an incubator at 37°c for 24 hours after which it is ready for use.

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI 600 003

EC Reg.No.ECR/270/Inst./TN/2013
Telephone No.044 25305301
Fax: 011 25363970

CERTIFICATE OF APPROVAL

To
Dr.R.Thirumurugan
Post Graduate in M.D. Micribiology
Madras Medical College
Chennai 600 003

Dear Dr.R.Thirumurugan,

The Institutional Ethics Committee has considered your request and approved your study titled " **AETIOLOGIES OF ACUTE UNDIFFERENTIATED FEBRILE ILLNESS IN ADULT PATIENTS IN A TERTIARY CARE HOSPITAL" NO.- 21052016**

The following members of Ethics Committee were present in the meeting hold on **03.05.2016** conducted at Madras Medical College, Chennai 3.

1. Dr.C.Rajendran, MD.,	:Chairperson
2. Dr.Isaac Christian Moses.,M.D.,Dean,MMC,Ch-3	:Deputy Chairperson
3. Prof.Sudha Seshayyan,MD., Vice Principal,MMC,Ch-3	: Member Secretary
4. Prof. B.Vasanth, M.D.,(Prof of Pharmacology)	: Member
5. Prof.P.Raghumani, M.S.,Prof ofSurgery, Inst. of Surgery	: Member
6. Prof. Md Ali, M.D., D.M.(Prof & HOD of MGE)	: Member
7. Prof. K.Ramadevi, M.D., (Director of Biochemistry)	: Member
8. Prof.M.Saraswathi,MD.,Director, Inst.of Path,MMC,Ch-3	: Member
9. Prof.Srinivasagalu,Director,Inst.of Int.Med.,MMC,Ch-3	: Member
10.Tmt.J.Rajalakshmi, JAO,MMC, Ch-3	: Lay Person
11.Thiru S.Govindasamy, BA.,BL,High Court,Chennai	: Lawyer
12.Tmt.Arnold Saulina, MA.,MSW.,	:Social Scientist

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

Member Secretary - Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE.
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

PROFORMA

- Name : IP NO:
- Age: Ward:
- Sex:
- Occupation:
- Address:

- Presenting complaints

- Personal history

- Past history

- Prior antibiotic therapy

- Microbiological investigation:
  - Direct examination -

- Culture : Bacterial culture / Antibiotic Sensitivity

- Serological Test

- Antibiotic sensitivity (if required)

INFORMATION SHEET

STUDY TITLE : AETIOLOGIES OF ACUTE UNDIFFERENTIATED FEBRILE ILLNESS IN ADULT PATIENTS IN A TERTIARY CARE HOSPITAL

INVESTIGATOR : **Dr. R. Thirumurugan,**
Post Graduate,
Institute of Microbiology,
Madras Medical College,
Chennai - 600003.

GUIDE : **Dr.Vanaja,**
Professor of Microbiology,
Institute of Microbiology,
Madras Medical College,
Chennai 600 003.

AUFI defined as fever less than 14 days of duration without any evidence of organ or systemic specific aetiology. AUFI also known as "Short febrile illness" or Acute fever or Acute febrile illness. Infectious disease are leading causes of morbidity and mortality in tropical countries like India. Acute undifferentiated febrile illness (UFI) is a common clinical entity in most of the hospitals.

The main etiological agents for AUFI includes malaria, Enteric fever, dengue, scrub typhus, [illegible]s, leptospirosis etc. In resource limited settings fever may be treated empirically or self treated due to lack of access to diagnostic tests. Thus knowledge of local prevalence of infection is critical in order to target clinical workup and treatment. Thus this study will be done to describe the aetiology of fever among adult patients in a tertiary care hospital. In case of any doubt please contact Dr. R. Thirumurugan, Cell :9894219971

CONSENT FORM

STUDY TITLE : AETIOLOGIES OF ACUTE UNDIFFERENTIATED FEBRILE ILLNESS IN ADULT PATIENTS IN A TERTIARY CARE HOSPITAL

I....., hereby give consent to participate in the study conducted by Dr. R.Thirumurugan, Post graduate at Institute of Microbiology, Madras Medical College, Chennai and to use my personal clinical data and the result of investigations for the purpose of analysis and to study the nature of the disease, I also give consent to give my blood for further investigations. I also learn that there is no additional risk in this study. I also give my consent for my investigator to publish the data in any forum or journal.

Signature/ Thumb impression Of the patient/ relative

Place

Date

Patient Name & Address:

Signature of the investigator:

Signature of guide

தகவல் படிவம்

ஆய்வு செய்யப்படும் தலைப்பு :

ஒரு மூன்றாம் நிலை பராமரிப்பு மருத்துவமனையில் 18 வயதுக்கு மேற்பட்டவர்களுக்கு வகைப்படுத்த இயலாத காய்ச்சல் இருக்கும் பட்சத்தில் அதற்கான காரணத்தை கண்டறிவது பற்றிய ஆய்வு.

ஆய்வாளர் : **மரு. ர. திருமுருகன்,**
முதுகலை பட்டப்படிப்பு மாணவர்,
நுண்ணுயிரியல் துறை,
சென்னை மருத்துவக் கல்லூரி,
சென்னை-600003.

வழிகாட்டி : மரு. R.வனஜா,
பேராசிரியர்,
நுண்ணுயிரியல் துறை,
சென்னை மருத்துவக் கல்லூரி,
சென்னை-600003.

காய்ச்சல் என்பது சாதாரணமாக அனைவருக்கும் ஏற்படக்கூடிய ஒரு அறிகுறியாகும். நான் 18 வயதுக்கு மேற்பட்டவர்களையும், 14 நாட்களுக்குள் வரை காய்ச்சல் உள்ளவர்களையும் ஆய்வு செய்ய உள்ளேன்.

காய்ச்சலை முன்னதாகவே கண்டுபிடித்து அதன் காரணத்தை கண்டறிவதால் சில தேவையற்ற பக்க விளைவுகளை தடுக்க முடியும் என்பதை வலியுறுத்த உள்ளேன்.

இந்த ஆய்வு முற்றிலும் தன்னார்வமிக்கது மற்றும் காய்ச்சல் உள்ளவர்கள் இந்த ஆய்விலிருந்து எந்த நேரத்திலும் விலகிக் கொள்ளலாம். இந்த ஆய்வில் நோயாளிகளுக்கு எந்த செலவும் இல்லை.

இந்த ஆய்வையொட்டி எந்த விதமான சந்தேகங்களுக்கும் விளக்கம் பெற பங்கேற்பாளர்களுக்கு உரிமை உள்ளது.

இந்த ஆய்வில் ஆகும் அதிகப்படியான செலவிற்கு நோயாளிகளிடமிருந்து பணம் பெற்றுக்கொள்ளப்படமாட்டாது.

இந்த ஆய்வின் முடிவுகள் இறுதியில் பிரசுரிக்கப்படும்.. இந்த ஆய்வை பற்றிய சந்தேகங்கள் முழுமையாக தங்களுக்கு விளக்கப்படும். தொடர்பு கொள்ள வேண்டியவர் மரு. ர.திருமுருகன், கைபேசி : 9894219971.

ஆய்வாளர் கையொப்பம்

தேதி :

பங்கேற்பாளர் கையொப்பம் /
இடதுகை பெருவிரல் ரேகை

தேதி :

MASTER CHART

S.No	Age (Years)	Sex	Duration of Fever (Days)	Rigger & Chills	Headache	Nausea & Vomiting	Joint Pain	Cough Dyspnea	Diarrhea & Loose Stools	Oliguria	Calf Muscles tenderness	Eschar
1	40	M	12	✓	-	✓	✓	-	-	-	-	-
2	30	M	10	✓								
3	26	M	5	✓		✓			✓			
4	26	M	7	✓		✓		✓	✓			
5	35	M	6	✓	✓	✓						
6	42	M	5	✓		✓		✓				
7	35	F	6	✓								
8	60	M	7	✓		✓						
9	35	M	6	✓		✓						
10	63	F	6	✓								
11	40	F	10	✓		✓						
12	33	M	10	✓				✓				
13	42	M	10	✓	✓	✓						
14	25	M	7			✓						
15	19	F	5	✓								
16	70	M	7	✓								
17	21	F	5		✓	✓						
18	19	M	6	✓	✓	✓						
19	18	M	6	✓		✓	✓					
20	41	M	10	✓		✓						
21	23	M	8									
22	22	M	7	✓								
23	30	F	7	✓					✓			
24	18	M	9	✓			✓					
25	18	M	7	✓		✓			✓			
26	18	F	8									✓
27	19	F	9									✓
28	23	M	7									✓
29	20	M	7									✓
30	18	F	7									✓
31	35	F	7	✓	✓	✓					✓	
32	28	M	7	✓	✓	✓						
33	30	M	7	✓		✓						

S.No	Age (Years)	Sex	Duration of Fever (Days)	Rigger & Chills	Headache	Nausea & Vomiting	Joint Pain	Cough Dyspnea	Diarrhea & Loose Stools	Oliguria	Calf Muscles tenderness	Eschar
34	18	M	7	✓	✓							
35	25	M	5	✓								
36	32	F	7	✓			✓					
37	19	F	10	✓		✓						
38	54	M	8	✓	✓			✓				
39	45	F	7	✓								
40	22	M	**5**	✓								
41	30	M	6	✓		✓						
42	23	M	6	✓		✓						
43	34	F	6	✓		✓						
44	23	M	7	✓					✓			
45	35	M	8	✓					✓	✓		
46	19	F	6	✓		✓						
47	65	M	7	✓				✓		✓		
48	23	F	10	✓	✓	✓						
49	21	M	7	✓	✓							
50	15	F	14	✓		✓						
51	45	M	12	✓	✓	✓			✓			
52	69	M	10	✓	✓	✓						
53	49	**F**	8	✓	✓		✓					
54	18	M	6	✓								
55	48	F	8	✓		✓						
56	34	**M**	7	✓		✓						
57	80	F	12	✓		✓						
58	19	M	10	✓		✓				✓		
59	45	F	10	✓	✓						✓	
60	25	M	9	✓				✓			✓	
61	21	M	8	✓		✓						
62	37	M	7	✓		✓						
63	32	M	7	✓		✓				✓		
64	19	F	6	✓		✓						
65	20	F	8	✓		✓						
66	18	M	7	✓		✓			✓			
67	19	M	6	✓		✓				✓		
68	22	F	8	✓								

S.No	Age (Years)	Sex	Duration of Fever (Days)	Rigger & Chills	Headache	Nausea & Vomiting	Joint Pain	Cough Dyspnea	Diarrhea & Loose Stools	Oliguria	Calf Muscles tenderness	Eschar
69	29	F	9	✓	✓							
70	38	F	7	✓	✓							
71	45	M	7	✓	✓	✓						
72	30	F	8	✓		✓						
73	19	F	7	✓	✓	✓						
74	35	M	9	✓		✓						
75	53	M	10	✓		✓						
76	18	M	8	✓	✓							
77	**25**	M	9	✓	✓	✓						
78	36	M	7	✓		✓						
79	65	F	7	✓				✓				
80	40	**F**	5	✓								
81	56	**F**	12									
82	19	**F**	10	✓								
83	45	F	8	✓		✓						
84	34	F	6	✓	✓	✓						
85	46	**F**	10	✓	✓							
86	25	M	7	✓	✓	✓						
87	50	F	5	✓	✓	✓						
88	65	M	14	✓					✓	✓		
89	**19**	**F**	7	✓								
90	39	M	6	✓								
91	18	**F**	7	✓	✓							
92	19	**F**	7	✓								
93	25	**F**	10	✓	✓	✓						
94	19	**M**	5	✓	✓					✓		
95	39	F	4	✓		✓		✓		✓		
96	37	F	5	✓		✓						
97	45	F	14	✓	✓	✓						
98	19	M	5	✓								
99	34	M	**5**	✓		✓				✓		
100	58	F	**7**	✓		✓						
101	23	F	7	✓		✓						
102	58	M	7	✓	✓	✓			✓			
103	34	M	7	✓		✓						

S.No	Age (Years)	Sex	Duration of Fever (Days)	Rigger & Chills	Headache	Nausea & Vomiting	Joint Pain	Cough Dyspnea	Diarrhea & Loose Stools	Oliguria	Calf Muscles tenderness	Eschar
104	33	M	14	✓	✓							
105	**36**	**F**	5	✓		✓						
106	22	M	5	✓		✓						
107	36	F	5	✓		✓				✓		
108	29	M	6	✓		✓						
109	57	M	7		✓	✓						
110	25	M	5	✓		✓			✓			
111	42	F	7	✓	✓							
112	**35**	**F**	10	✓	✓			✓	✓			
113	50	F	7									
114	33	F	4									
115	20	**F**	4	✓	✓							
116	19	**F**	5	✓	✓	✓						
117	18	**M**	8	✓	✓	✓						
118	24	**M**	7	✓		✓						
119	33	**M**	4	✓								
120	40	F	10	✓		✓						
121	26	**M**	7	✓								
122	25	F	7	✓	✓	✓						
123	65	M	7	✓	✓							
124	19	M	7	✓	✓	✓						
125	28	F	7	✓	✓	✓						
126	18	M	8	✓		✓			✓			
127	33	**F**	14	✓	✓	✓			✓			
128	45	F	7	✓					✓			
129	23	**F**	7	✓		✓						
130	65	**M**	7	✓				✓		✓		
131	57	M	6	✓		✓						
132	28	M	10	✓		✓				✓		
133	30	M	7									
134	21	F	7	✓	✓	✓						
135	18	M	5	✓		✓						
136	24	M	7									
137	29	M	9									
138	35	F	10									

S.No	Rash	Jaundice	Arthralgia	Altered Sensorium	Abd. Pain	Bleeding Manifes.	Retroarbital Pain	Haemata mesis	WBC Count	Platelet count	S. Bilirubin	SGOT IU/L	SGPT IU/L
1										25000		188	129
2										30000		212	130
3					✓								
4					✓				4x10 3ul				
5		✓								4000	2	156	128
6					✓				4x10 3ul				
7		✓							4x10 3ul	3000	2.4	144	112
8		✓		✓							2.6		
9										4500			
10										4000			
11						✓		✓		26000			
12		✓											
13					✓				3x10 3ul				
14										3500			
15			✓	✓			✓						
16			✓							4000			
17										3500			
18			✓	✓						4500			
19										4000			
20													
21										30000	3.2		
22									Anemia		3		
23		✓											
24			✓				✓				2.4		
25		✓			✓						2.6		
26	✓	✓							3.7x10		2.8		
27	✓										2.6		
28	✓										2.4		
29	✓										2		
30	✓										1.4		
31											2.8	176	124
32									Anemia				
33					✓	✓					1.8		
34				✓	✓	✓					2.6	12	14
35													
36			✓				✓		4.18x10				
37										20000			
38										19000			
39													
40			✓				✓			2000		86	72

S.No	Rash	Jaundice	Arthralgia	Altered Sensorium	Abd. Pain	Bleeding Manifes.	Retroarbital Pain	Haemata mesis	WBC Count	Platelet count	S. Bilirubin	SGOT IU/L	SGPT IU/L
41													
42					✓	✓			3.5x10				
43										4000			
44					✓					2000			
45					✓					3000			
46									3.5x10				
47										2500			
48									Anemia				
49										4000			
50									Anemia				
51					✓					3000		142	126
52						✓							
53		✓								19000			
54				✓						30000			
55										4000			
56		✓											
57										2000	1.8		
58		✓			✓				3.5x10		1.9	16	12
59			✓										
60									5.5x10				
61					✓					20000		24	14
62				✓	✓					30000		38	16
63					✓							47	14
64						✓		✓					
65												128	119
66					✓				Anemia				
67					✓							114	105
68					✓					19000		88	76
69					✓	✓		✓					
70												9478	
71						✓		✓		26000			
72					✓					30000			
73					✓							84	76
74				✓	✓	✓		✓					
75					✓								
76					✓			✓		20000		104	94
77	✓				✓			✓	4.5x10				
78						✓		✓		39000		124	112
79													
80		✓							4.3x10		1.9		

S.No	Rash	Jaundice	Arthralgia	Altered Sensorium	Abd. Pain	Bleeding Manifes.	Retroarbital Pain	Haemata mesis	WBC Count	Platelet count	S. Bilirubin	SGOT IU/L	SGPT IU/L
81		✓							3.5		2.4		
82		✓							4.5		2.2	143	109
83									anemia				
84										40000			
85		✓									2.2		
86										18000		153	114
87													
88				✓	✓				4x10				
89	✓	✓									1.9		
90													
91		✓								20000	2.4		
92		✓			✓						2.6		
93		✓									1.2	109	98
94		✓									1.8		
95					✓						2.4		
96					✓				Anemia				
97						✓		✓		18000		146	112
98				✓	✓					19000			
99			✓										
100			✓		✓		✓			30000			
101					✓								
102										45000			
103				✓	✓								
104					✓			✓				164	148
105	✓	✓									2.5		
106					✓								
107						✓		✓		20000			
108					✓					22000			
109						✓		✓		25000			
110										20000			
111													
112	✓	✓									2.6		
113					✓	✓					2.4		
114						✓		✓			2.9		
115		✓									3.6		
116		✓									2.8		
117		✓			✓						1.9		
118		✓									2.4		
119		✓									3.2		
120													

S.No	Rash	Jaundice	Arthralgia	Altered Sensorium	Abd. Pain	Bleeding Manifes.	Retroarbital Pain	Haemata mesis	WBC Count	Platelet count	S. Bilirubin	SGOT IU/L	SGPT IU/L
121		✓									1.9		
122											2.9		
123					✓	✓		✓		20000			
124					✓			✓					
125										20000			
126					✓				4.2				
127		✓			✓								
128					✓				4.3		1.9		
129		✓				✓		✓			4		
130		✓									3.7		
131					✓			✓					
132					✓	✓		✓		19000			
133					✓	✓		✓		20000			
134						✓				20000			
135									Anemia				
136					✓			✓		18000			
137													
138													
139													
140					✓	✓		✓	14.3x10				
141													
142					✓				4.5x10				
143			✓										
144					✓			✓					
145													
146													
147													
148					✓			✓	3.5x10				
149													
150													

S.No	Age (Years)	Sex	Duration of Fever (Days)	Rigger & Chills	Headache	Nausea & Vomiting	Joint Pain	Cough Dyspnea	Diarrhea & Loose Stools	Oliguria	Calf Muscles tenderness	Eschar
139	24	M	7									
140	43	F	5	✓		✓			✓			
141	19	M	5	✓		✓				✓	✓	
142	21	M	5	✓	✓				✓			
143	18	M	**7**	✓			✓					✓
144	19	M	8	✓		✓						
145	19	F	5	✓				✓				✓
146	25	M	5	✓	✓						✓	
147	28	F	6	✓	✓			✓				
148	23	M	7	✓	✓	✓			✓	✓		
149	33	M	7	✓				✓				
150	42	F	7	✓		✓						

S.No	Urine Albumin	Blood Culture	Peripheral Smear (MP)	Malarial Ag	Dengue Ns1 Ag	Dengue IgM ELISA	Chickungunya IgM ELISA	MSAT	Widal	Scrub TyPhus	Un classified
1	-	-	-	-	Positive	Positive	-	-	-	-	-
2	-	-	-	-	Positive	Positive	-	-	-	-	-
3	-	-	-	-	Positive		-	-	-	-	-
4	-	-	-	-	-	-	-	-	Positive	-	-
5	-	-	-	-	-	-	-	Positive2+		-	-
6	-	-	-	-	-	-	-		Positive	-	-
7	-	-	-	-	-	-	-	Positive2+	-	-	-
8	-	-	-	-	-	-	-	Positive2+	-	-	-
9	-	-	-	-	Positive	-	-		-	-	-
10	-	-	-	-	Positive	-	-		-	-	-
11	-	-	-	-	Positive	-	-		-	-	-
12	-	-	-	-	-	-	-	Positive2+	-	-	-
13	-	-	Positive	-	-	-	-	-	-	-	-
14	-	-		-	-	Positive	-	-	-	-	-
15	-	-		-	-	-	Positive	-	-	-	-
16	-	-	Positive	-	-	-	-	-	-	-	-
17	-	Positive	-	-	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-	-	-	Positive	
19	-	-	-	-	-	-	Positive	-	-		
20	-	-	-	-	-	-	-	-	-		
21	-	-	-	-	-	Positive	-	-	-		
22	-	-	Positive	Positive			-	-	-		✓
23	-	-	-	-	-	-	-	Positive2+	-	-	-
24	-	-	-	-	-	-	Positive		-	-	-
25	-	-	-	-	-	-	-		-	-	-

S.No	Urine Albumin	Blood Culture	Peripheral Smear (MP)	Malarial Ag	Dengue Ns1 Ag	Dengue IgM ELISA	Chickungunya IgM ELISA	MSAT	Widal	Scrub TyPhus	Un classified
26	-	-	-	-	-	-	-	Positive2+	-	-	-
27	-	-	-	-	-	-	-	-	-	Positive	-
28	-	-	-	-	-	-	-	-	-	Positive	-
29	-	-	-	-	-	-	-	-	-	Positive	-
30	-	-	-	-	-	-	-	-	-	Positive	-
31	-	-	-	-	-	-	-	-	-	-	-
32	-	-	Positive	Positive			-	-	-	-	-
33	-	-	-	-	-	Positive	-	-	-	-	-
34	-	Positive	-	-	-		-	-	-	-	-
35	-	-	-	-	Positive	Positive	-	-	-	-	-
36	-	-	-	-	-	-	-	-	-	-	✓
37	-	-	-	-	-	-	Positive	-	-	-	
38	-	-	-	-	-	-	-	-	-	-	✓
39	-	-	-	-	Positive	-	-	-	-	-	-
40	-	-	-	-	Positive	Positive	-	-	-	-	-
41	-	-	-	-	-	-	Positive	-	-	-	-
42	-	-	-	-	-	-	-	-	Positive	-	-
43	-	-	-	-	-	Positive	-	-		-	-
44	-	-	-	-	-	Positive	-	-		-	-
45	-	-	-	-	-		-	-	Positive	-	-
46	-	-	-	-	-		-	-	Positive	-	-
47	-	-	-	-	-	Positive	-	-	-	-	-
48	-	-	Positive	Positive	-	-	-	-	-	-	-
49	-	-	-	-	-	-	-	Positive2+	-	-	-
50	-	-	Positive			-	-	-	-	-	-
51	-		-	-	Positive	-	-	-	-	-	-
52		Positive	-	-		-	-	-	-	-	-
53	-	-	-	-	Positive	-	-	-	-	-	-
54	-	-	-	-		Positive					

S.No	Urine Albumin	Blood Culture	Peripheral Smear (MP)	Malarial Ag	Dengue Ns1 Ag	Dengue IgM ELISA	Chickungunya IgM ELISA	MSAT	Widal	Scrub TyPhus	Un classified
55	-	-	-	-		Positive					
56	-	-	-	-				Positive2+			
57	-	-	-	-							✓
58	-	-	-	-				Positive2+		Positive	
59	-	-	-	-							✓
60	-	-	-	-							✓
61			-	-	Positive	Positive					
62			-	-		Positive					
63											✓
64											✓
65											✓
66											
67											
68						Positive					
69											✓
70											✓
71						Positive					
72					Positive	Positive					
73											✓
74											✓
75											✓
76						Positive					
77			Positive	Positive							
78						Positive					
79											✓
80								Positive2+			
81	Positive							Positive2+			

S.No	Urine Albumin	Blood Culture	Peripheral Smear (MP)	Malarial Ag	Dengue Ns1 Ag	Dengue IgM ELISA	Chickungunya IgM ELISA	MSAT	Widal	Scrub TyPhus	Un classified
82	Positive							Positive2+			
83			Positive	Positive							
84						Positive					
85								Positive2+			
86					Positive	Positive					
87											✓
88		Positive									
89	Positive							Positive2+			
90	Positive										✓
91						Positive					
92	Positive							Positive2+			
93											✓
94								Positive2+			
95								Positive2+			
96			Positive	Positive							
97						Positive					
98					Positive	Positive					
99											✓
100							Positive				
101											✓
102									Positive		
103											✓
104											✓
105								Positive2+			

S.No	Urine Albumin	Blood Culture	Peripheral Smear (MP)	Malarial Ag	Dengue Ns1 Ag	Dengue IgM ELISA	Chickungunya IgM ELISA	MSAT	Widal	Scrub TyPhus	Un classified
106											✓
107						Positive					
108						Positive					
109								Positive2+			
110						Positive					
111											✓
112								Positive2+			
113								Positive2+			
114								Positive3+			
115											
116								Positive2+			
117								Positive2+			
118								Positive2+			
119								Positive2+			
120		Positive									
121								Positive2+			
122								Positive2+			
123					Positive	Positive					
124											✓
125					Positive	Positive					
126											

S.No	Urine Albumin	Blood Culture	Peripheral Smear (MP)	Malarial Ag	Dengue Ns1 Ag	Dengue IgM ELISA	Chickungunya IgM ELISA	MSAT	Widal	Scrub TyPhus	Un classified
127								Positive2+			
128									Positive		
129								Positive2+			
130								Positive2+			
131											✓
132						Positive					
133						Positive					
134						Positive					
135			Positive								
136						Positive					
137											✓
138											✓
139											✓
140											✓
141											✓
142									Positive		
143										Positive	
144											✓
145										Positive	
146											✓
147											✓
148											✓
149											✓
150											✓