DISSERTATION ON

A CLINICOMICROBIOLOGICAL STUDY ON INFECTIVE CAUSES OF ACUTE FEBRILE ILLNESS

Dissertation submitted in partial fulfillment of the

Requirement for the award of the Degree of

M.D. MICROBIOLOGY (BRANCH IV)

TRICHY SRM MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTRE

IRUNGALUR, TRICHY- 621 105

Affiliated To

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

CHENNAI, TAMILNADU

CERTIFICATE

This is to certify that the dissertation entitled, **"A CLINICOMICROBIOLOGICAL STUDY ON INFECTIVE CAUSES OF ACUTE FEBRILE ILLNESS"** by Dr.A.S.Sathesini Priya, Post graduate in Microbiology (2017-2020), is a bonafide research work carried out under our direct supervision and guidance and is submitted to The Tamilnadu Dr. M.G.R. Medical University, Chennai, for M.D. Degree Examination in Microbiology, Branch IV, to be held in May 2020.

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DECLARATION

I solemnly declare that the dissertation titled **"A CLINICOMICROBIOLOGICAL STUDY ON INFECTIVE CAUSES OF ACUTE FEBRILE ILLNESS"** is bonafide record of work done by me during the period of January to December 2018, under the guidance of Professor and HOD DR.A.UMA, M.D., Department of Microbiology, Trichy SRM Medical College Hospital and Research Institute, Trichy.

The dissertation is submitted to The Tamil Nadu Dr.M.G.R Medical University in partial fulfillment of the requirement for the award of M.D Degree (Branch IV) in Microbiology.

Place: Trichy

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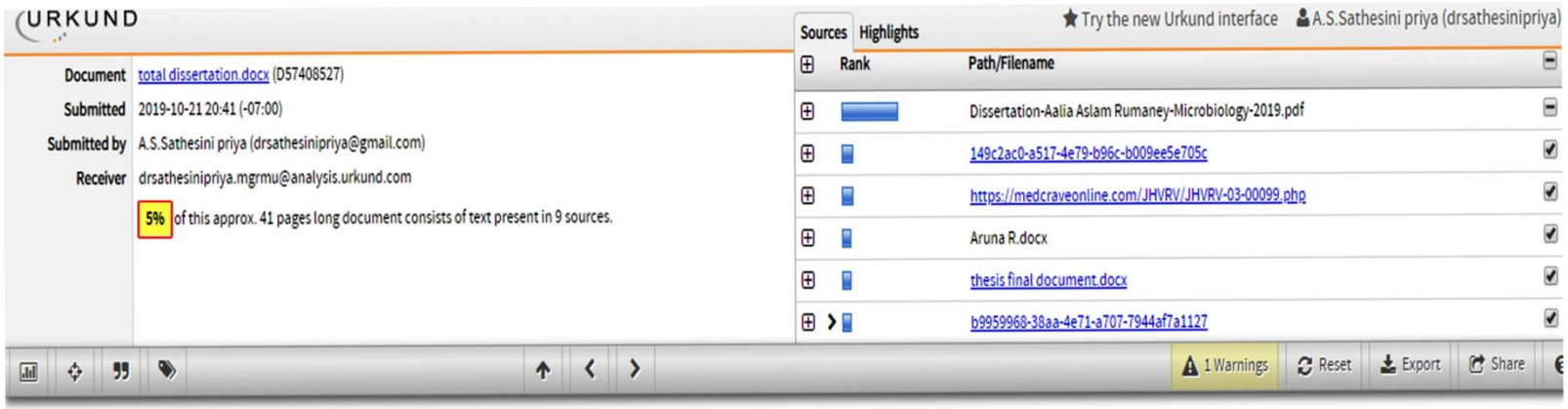

1.0. INTRODUCTION:

Fever has been the cornerstone in the field of medicine and one of the reliable marker of many different diseases. It is otherwise called as pyrexia which means 'fire' in Greek. The term febrile was derived from the Latin word 'Febris' which means fire. The temperature of the body can be affected by both environmental and biological factors such as age, gender, ethnicity, time of the day, anatomical site and physical activity [1]. Our body temperature is at its highest at about 6 pm and usually at its lowest at around 3 am [1]. An elevated body temperature (pyrexia/fever) is one of the effective ways by which the immune system combats an infection. Normally, elevated body temperature helps the immune system to resolve an infection. However, there may be a peak in rise of temperature at times where the fever could be serious and may result in severe complications [1]. Fever plays a pivotal role in the development of inflammation and is one of the four cardinal features of inflammation. When measured orally, a rise in a.m temperature of <37.2℃ or a p.m temperature of <37.7℃ would determine fever [2]. Fevers can be classified based on the duration into three, namely- acute, subacute and chronic [1]. Acute undifferentiated fever(AUF) is also given the term "acute febrile illness", "acute fever" or "short febrile illness" and are the synonyms for AUFI. The cases of fever with proven diagnosis are given the term "Diagnosed AUF" and those with defied diagnosis are given the term "Undiagnosed Undifferentiated fever" [3]. Figure 1: Classification of fever

Febrile response Early rapid response depends on peripherally synthesised PGE2 synthesised from lung and liver delayed late response depends on centrally synthesised PGE2

Total study subjects - 500 Subjects satisfied inclusion criteria -270 Subjects included - 230 and diseases diagnosed - 235 Dengue-132*, Typhoid-5, Enteric fever-72, Malaria-13, Scrub typhus-7, Leptospirosis-5

ANNEXURE- I

TRICHY SRM MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTRE
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Email: researchcmchrc@gmail.com; Phone: 0431-2258691, 2258817

Ref. No: 108/ IEC / TSRMMCH&RC/ ME-1 **Date: 05.12.2017**

INSTITUTIONAL ETHICS COMMITTEE CERTIFICATE

Sub: Approval of research work - Issuance of IEC certificate - reg.

The research proposal submitted and presented by **Dr. A. S. Sathesini Priya, I year Postgraduate, Department of Microbiology**, Chennai Medical College Hospital and Research Centre, was discussed by the Institutional Ethics Committee of CMCH&RC. The committee has **approved** the research project subjected to the following regulations.

Title of the Research work: **A Clinicomicrobiological study on infective cause of Acute febrile illness.**

a. She should abide the guidelines of this Institutional Ethics Committee.
b. She should not deviate from the proposal submitted.
c. She has to inform IEC if any deviations/ modifications whenever necessary.
d. She should carry out the project within the stipulated period and if extension needed, she has to inform IEC.
e. She should get informed consent from the subjects/ patients of the study group.
f. She should not claim any monetary support from IEC.
g. She should submit a project completion report to the Member Secretary, IEC within one month after completion.
h. She may use this approval letter for applying to funding agencies and publications.
i. **IEC comments: Approved.**

5/12/2017

Member Secretary
[Dr. S.D. Nalinakumari]

To:

Dr. A. S. Sathesini Priya,
I year Postgraduate,
Department of Microbiology.

INSTITUTIONAL ETHICS COMMITTEE
Date 15.12.17

Member Secretary
INSTITUTIONAL ETHICS COMMITTEE

ANNEXURE- II

CASE STUDY PROFORMA

1	IP/OP No.	
2	Case no:	
3	Mob no:	
4	D.O.A:	
5	Name	
6	Age / Gender	
7	Chief/Present complaint	
a)duration & onset		
b) Pattern of fever		
c)aggravating/relieving fact:		
d)Associated symptoms		Chills, rigors/Headache/night sweats/ nausea/ vomiting/ sorethroat/ dyspnea/ cough,expectoration/ loose stools/ abd pain/burning micturation/jaundice/ rash/arthralgia/ myalgia/ altered sensorium/ bleeding manisfestation / retro orbital pain
8	Past history	1. any other illness 2. any surgeries, if yes, type of surgery? 3. any transplantation/any immunosuppressive drugs ? 4. any minor procedures? Bleeding ? 5. any trauma/ RTA – time/ place/type
9	Drug history / allergy history	Yes/no, if yes, a)duration of illness b)type of medication blood transfusion:
10	Family history	Familial disease / any infection / epidemic disease
11	Personal history	1)diet: 2)sleep habits: 3)DM-yes/no, if yes, a)duration b)type 4)HTN-yes/no, if yes, a)duration b)type 5) any illicit drug- a)duration b)type
12	Social history	
a)	Locality	Urban/semiurban/rural
b)	Educational status	noneducated/elementary/high school/ hr.sec/ graduated/post graduate
c)	Occupational status	Labourer/semiskilled/skilled/professionals/Unemployed Name of the occupation:
d)	Socio economic status	Poor/middle class/upper middle class high income group
e)	Marital status	Married/unmarried/living alone
f)	Home condition	
g)	Sanitation status	Home:
		Surrounding:
h)	Animal handling	Yes/ no, has pets?
i)	Vector or rodents	Type:
		Preventive step:
j)	Drinking water	OHT(chlorinated)/pond/purified like RO/bore well/well(open

		type/close type)
k)	Consumption of unpasteurized milk or milk products:	
13	Other relevant history	Vaccination history: Travel / Sexual history:
14	Generalexamination:	Consciousness / orientation
	Signs:	Pallor / icterus /cyanosis / clubbing/ edema/ rashes/ lymphadenopathy
15	**Systemic ex:** a)CVS	
	b)RS	
	c)GIT	
	d)CNS	
	e)Others	

Sample collection:

1	Date of sample collection	
2	Type of sample collected & type of test	
4	Testing technique	
5	Laboratory report	
	• CBC report	
	• URINE ANALYSIS	
	• LFT	
	• RFT & ELECTROLYTE	
	• CRP	
	• HIV	
	• HBsAG	
	• HCV	
	• WIDAL	
	• DENGUE	
	• MALARIA	
	• BRUCELLA	
	• SCRUB TYPHUS	
	• LEPTOSPIROSIS	
	• CHIKUNGUNYA	
6	Date Of Discharge	
7	Condition On Discharge	Improved/ AMA/Death

ANNEXURE- III

ஒப்புதல்கடிதம்

பங்கேற்பாளரின்பெயர்:

ஆராய்ச்சியின்தலைப்பு:

இந்த ஆராய்ச்சியைப் பற்றிய அனைத்து விவரங்களும் எனக்கு எழுத்து மூலமாகவும் வாய்மொழியாகவும் எனது தாய்மொழியில் விவரிக்கப்பட்டது. இவ்வாராய்ச்சியைப் பற்றி முழுமையாகப் புரிந்து கொண்டேன்.

இவ்வாராய்ச்சியைப் பற்றிய கேள்விகளைஎழுப்ப எனக்கு வாய்ப்பு கொடுக்கப்பட்டது. இவ்வாராய்ச்சியில் நான் பங்கு பெறுவது எனது சொந்த விருப்பத்தை பொறுத்தது என்றும்,இந்த சோதனைக்கு நான் முழுதும் சம்மதிக்கிறேன் என்றும் உறுதியளிக்கிறேன்.இதற்கு நான்எந்தவித தடையும் இடையூறும் செய்யமாட்டேன் என்று சுய நினைவுடன்உறுதியளிக்கிறேன். இவ்வாராய்ச்சிக்கு என்னை உட்படுத்த பணம் எதுவும்பெறவில்லை என்றும் உறுதியளிக்கிறேன்

பங்கேற்பாளர் கையொப்பம்:

தேதி:

CERTIFICATE – II

This is to certify that this dissertation work titled **"A CLINICOMICROBIOLOGICAL STUDY ON INFECTIVE CAUSES OF ACUTE FEBRILE ILLNESS"** of the candidate **Dr.A.S.Sathesini Priya** with registration Number 201714602 is for the award of **M.D.MICROBIOLOGY** in the branch of IV. I personally verified the urkund.com website for the purpose of plagiarism Check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows 5**%** percentage of plagiarism in the dissertation.

Guide & Supervisor signature with Seal.

ACKNOWLEDGEMENT

I humbly submit this work to **ALMIGHTY**, who has given me the strength, endurance and ability to overcome the difficulties encountered in the process of compilation of my dissertation work and my family members for their everlasting support, encouragement and heartfelt blessings throughout the study.

I wish to express my sincere thanks to our **DEAN Dr. A. Jesudoss M.S., D.L.O.,** Trichy SRM Medical College Hospital and Research Centre, Trichy**,** for permitting me to use the resources of this Institution for my study.

Firstly, I would like to express my sincere gratitude to **Dr. A. Uma** my beloved **Prof & Head of Microbiology**, Trichy SRM Medical College Hospital and Research Centre for her timely suggestions and valuable guidance during my work. She has a great role in improving my ability to analyze the study.

I would like to whole heartedly thank **Dr.Thirumalaikolundu Subramanian,** Professor of Medicine for his valuable suggestion for completion of my dissertation work.

I express my thanks and heartfelt gratitude to my co guide **Dr.G.Vazhavandal,** Associate Professor, Department of Microbiology, Trichy

SRM Medical College Hospital and Research Centre, for her valuable technical support and constant encouragement to complete this study.

I would like to thank all the Assistant Professors of the Department of Microbiology, Trichy SRM Medical College Hospital and Research Centre, for their voluntary valuable assistance and encouragement during the study period.

Special thanks to **Dr. Saro Thanga Sangeetha**, my fellow post graduate, for her immense support, encouragement and also for helping me in my works. I would also wish to thank my junior postgraduates for their help.

I would like to thank all staff of Department of Microbiology, Trichy SRM Medical College Hospital and Research Centre.

CONTENTS

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1.0. **INTRODUCTION:**

Fever has been the cornerstone in the fieldof medicineand one of the reliable marker of many different diseases. It is otherwise called as pyrexia which means ‘fire’ in Greek. The term febrile was derived from the Latin word ‘Febris’ which means fire. The temperature of the body can be affected by both environmental and biological factors such as age, gender, ethnicity, time of the day, anatomical site and physical activity [1].

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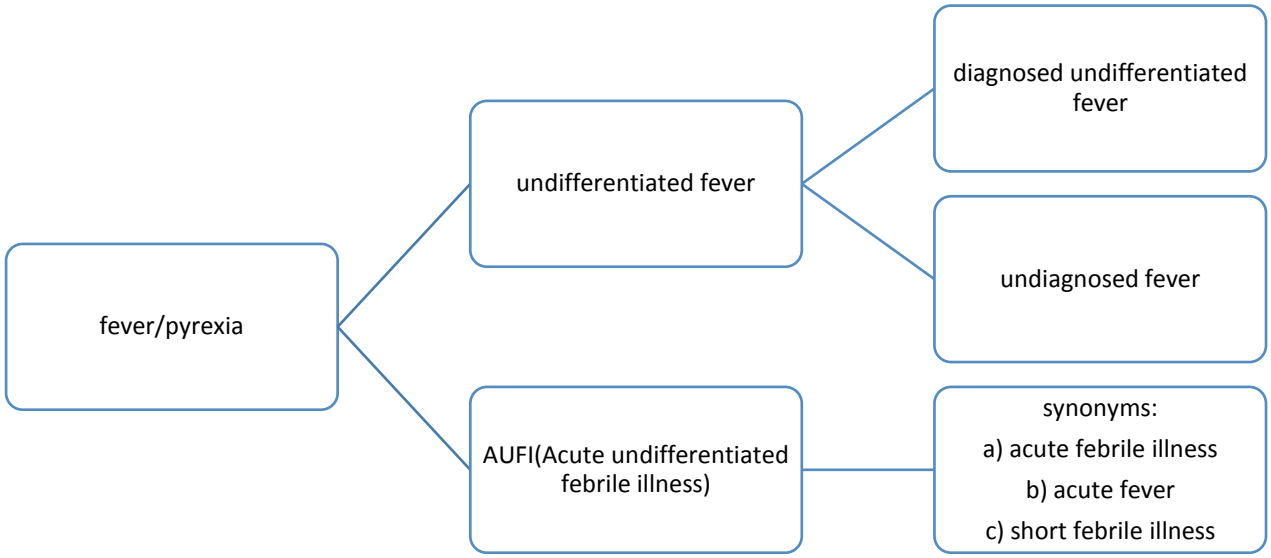
Fever plays a pivotal role in the development of inflammation and is one of the four cardinal features of inflammation. When measured orally, a rise in a.m temperature of $>37.2^0$C or a p.m temperature of $>37.7^0$Cwould determine fever [2]. Fevers can be classified based on the duration into three, namely- acute, subacute and chronic [1]. Acute undifferentiated fever(AUF) is also given the term “acute febrile illness”, “acute fever” or “short febrile illness” and are the synonyms for AUFI. The cases of fever with proven diagnosis are given the term “Diagnosed AUF” and those with defied diagnosis are given the term “Undiagnosed Undifferentiated fever” [3].

Figure 1: Classification of fever



Acute febrile illness (AFI) is described asany illness along with fever of two weeks or shorter in duration, whose onset israpid,caused by diverse pathogens with noevidence of organor system-specificetiology [5]. Acute febrile illness is also called as Acute undifferentiated febrile illnesses (AUFIs)and cause considerable morbidity, mortality and economic burden specially in developing tropical nations [6].

Fever can be due to many causes like infections, autoimmune disorders like rheumatoid arthritis, malignancies, overexposure to sunlight or sunburn, heat stroke due to prolonged strenuous exercise or exposure to high temperatures, dehydration, silicosis, amphetamine abuse, atrophine toxicity, drug-induced, alcohol withdrawal syndrome,etc [4].

In the Western world, AUFIis mostly due to self-limited viral conditions. In thedeveloping world, the differential diagnosis for AUFImostly includes potentially significant illnesses such as Malaria, Dengue Fever, Enteric Fever, Leptospirosis,

Rickettsiosis, Hantavirus and Japanese Encephalitis [7]. These infections have identical clinical signs and symptoms, and the correct diagnosis can be obtained by using pathogen specific diagnostic tests [8].

There is a variation seen in the causation of the AUFIbased on the season and the geographic location [9]. The emergence and reemergence of infections in tropical regions like Tamil Nadumay also pivot on the factors like Climate variation, over population and urbanization [10]

Arthropod-borne diseases likeDengue fever and Malaria are endemic in many parts of India especially during monsoon and post-monsoon season. Zoonotic infections like Leptospirosis and Scrub typhus are rampantly present in regions of heavy monsoon and agrarian way of occupation [11]. Dengue fever constitutes about 14% during 2008 and 48% among all fever cases in a population-based study conducted in rural South India and urban North India respectively [12,13].

Joshi et al. made a study in Central India on AUFI and identified that 88% belong to non malarial causes while the percentile was 17.1% according to another South Indian study [12,13].The commonest bloodstream bacterial infection was Salmonella, which was causative for 1/10th of AFI in a study conducted in North India [12]. Leptospirosis accounted upto 1/3rd of AUFI in an old estimate and is much prevalent in the coastal regions especially Chennai and Kolkatta [12]

Confirmation of etiology remains a challenge and it requires careful evaluation of clinical findings related to fever. The prevention of complications and the reduction in mortality and morbidity among the febrile cases solely depends on the

good correlation between clinical findings and confirmatory diagnostic tests, appropriate laboratory parameters and epidemiology of the illness. This study was conducted to find the association between the infective etiology and clinical presentation along with the importance of laboratory markers among the acute febrile patients.

2.0. AIMS AND OBJECTIVES:

1. To elicit the clinical and laboratory aspects of fever
2. To find out the causes of Acute febrile illness
3. To highlight on the responsibilities of microbiologists in the disease containment.

3.0. REVIEW OF LITERATURE:

The elevated body temperature is described aspyrexia or fever. Two terminologies are often described while handling cases of fever are hypothermia (core temperature below 35°C) and hyperthermia (core temperature above 40°C). It happens due to inundation of cooling mechanisms and alteration in the thermoregulation of the body.

Pyrexia denotes the elevation in body temperature above reference range for the species, where a cause is not inferred. Fever is the term which represents an increase in the body temperature which results due to an upward alteration in the body thermostat [14].Pyrexia of unknown origin (PUO) is defined as fever >38.3 °C (101°F) on several occasions,usually interpreted as atleast three, duration of more than three weeks and without any diagnosis after one week of inpatient investigation [15]. The cause of PUO are tabulated in Table 1. Historical aspects related to fever are described in Table 2.

Table 1: Causes of Pyrexia of unknown origin [15]

S.no	Category	Specific agents
1	Bacterial	• Tuberculosis • Coxiella burnetti • Brucellosis • Occult abscess
2	Viral	• Epstein-barr virus • Cytomegalovirus • Human immunodeficiency virus
3	Parasitic	• Malaria • Toxoplasmosis
4	Fungal	• Histoplasmosis

5	Inflammatory	• Giant cell arthritis • Adult still's disease • Systemic lupus erythematosus • Polyarteritis nodosa
6	Neoplastic	• Non-hodgkin's lymphoma • Leukemia • Hepatocellular carcinoma
7	Miscellaneous	• Drugs • Cirrhosis • Pulmonary embolism • Sarcoidosis

Table 2: Historical events of Pyrexia[15]

S.No	Contributors	Period of time	Contributions
1		Sixth century B.C	The Akkadian cuneiform inscriptions depicted fever and the local signs of inflammation in the form of ancient Sumerian pictograms of a flaming brazier.
2	Hippocratic physicians	370 BC	postulated a theory that fever was a result of excess of yellow bile, as most febrile patients has associated jaundice
3	William Harvey	Eighteenth century	Believed that fever was a result of the friction between the blood and vascular system
4	Claude Bernard	Eighteenth century	metabolic processes taking place in our body was responsible for the establishment of fever and body heat
5	Carl August	1868	Introduced the measurement of mean healthy

	Wunderlich		body temperature in humans and also proposed the concept of normal body temperature and diurnal variation of body temperature
6	Atharvaveda	Second millennium BC	Referred Jwara with the name Takman (son of God Varuna)

The ancient known written reference to fever was found in the Akkadian cuneiform inscriptions from the sixth century B.C, in the form of ancient Sumerian pictograms of a flaming brazier that represented fever and the local signs of inflammation[15].Later, Hippocratic physicians postulated a theory that fever was a result of excess of yellow bile, as most febrile patients had associated jaundice. Following Harvey's discovery of blood circulation, it was believed that, fever was a result of the friction between the blood and vascular system. The great works of Claude Bernard, a French physiologist, revealed that the metabolic processes taking place in our body was responsible for the establishment of fever and body heat. The measurement of mean healthy body temperature in humans and the concept of normal body temperature and diurnal variation of body temperature was given by Wunderlich[15].

Mechanism:

The febrile response is a complex physiologic reaction to disease, involving a cytokine-mediated rise in the core temperature, generation of acute phase reactants

and activation of numerous physiologic, endocrinologic and immunologic systems [15].

The balance in body temperature is tightly maintained at a constant range-thermal balance point [17]which is carried out by the process of thermoregulation, by various independent thermoeffector loops, each owning independent afferent and efferent branches [18,19]. The major thermoregulatory centre in the CNS is the preoptic region of the anterior hypothalamus, where peripheral and centrally generated signals are processed. [18,19]

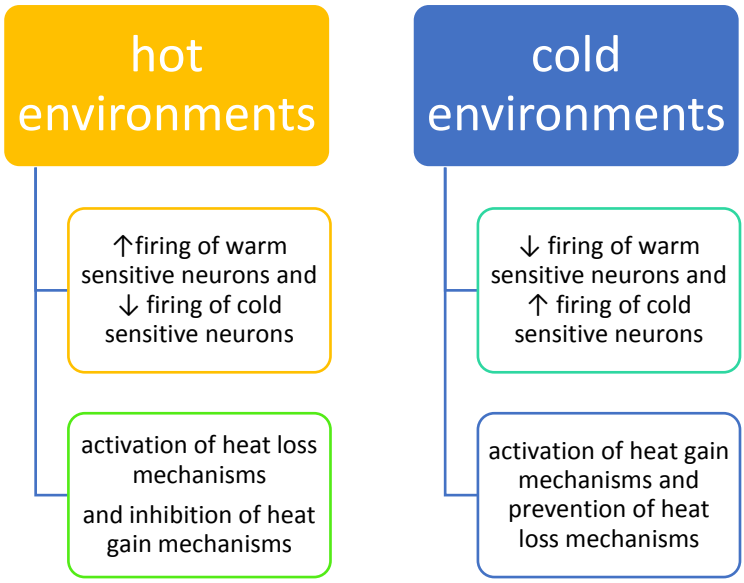


Figure no. 2: Process of Thermoregulation

3.1. The role of pyrogens and cryogens – fever

The balance in the interactivity between pyrogens and cyrogens decides the height and course of the febrile response to any immune challenge [17]. Pyrogens are categorized into two groups based on their site of production.

1. exogenous (produced outside the host)
2. endogenous (produced within the host)

Exogenous pyrogens consists of eitherpart of or whole micro-organisms or products of microorganisms like the toxins. Examples include lipopolysaccharide (LPS), muramyl dipeptidase, and enterotoxins of *Staphylococcus aureus* and group A and B *Streptococcus*cumulativelynamed superantigens [20]. Endogenous pyrogens are produced either due to induction by exogenous pyrogens or without their induction. On induction they are secreted by immune cells such as neutrophils, macrophages lymphocytes and also by the endothelial cells, astrocytes and glial cells. Examples include pyrogenic cytokines like interleukins (IL) 6, IL-1, interferon gamma (INF) and ciliary neurotropic factor (CNTF) and tumour necrosis factor (TNF) [21,22]. Certain endogenous substances such as antigen-antibody complexes, inflammatory bile acids, complements and various lymphocyte derived molecules act as pyrogens without induction by exogenous pyrogens [21]

Cryogens produce endogenous antipyretic systems which play a major role in preventing the host from the destructive consequences of uncontrolled fever. They consists of anti-inflammatory cytokines, neuroendocrine products, hormones and cytochrome P-450 [21,23,24].

3.2. The fever pathways

Both exogenous and endogenous pyrogens stimulate fever signals which readjust the thermoregulatory circuitry through two basic pathways - the humoral and neural pathways.[25-27]

The humoral pathway

Fever production is mediated by two pathways:

1)humoral pathway

2) neural pathway

In humoral pathway,fever signals are carried by pathogen associated molecular patterns(PAMPS) or by pyrogenic cytokines and these pyrogenic particles are usually secreted by cell types namely myeloid and epitheloid cell types. Examples include IL-1, IL-6 and TNF. They bind to the Toll like receptors – 4, which are located on the fenestrated capillaries of the circumventricular organ in the Blood brain barrier. The arachidonic acid pathway in the cytoplasmic membrane gets activated and Prostaglandin E2 (PGE2) is released. They specifically binds to the PGE2 receptors in the preoptic area. There is activation of thermal neurons in the anterior hypothalamus to higher thermal balance point[21,28-30]. The humoral pathway is depicted ina pictorial form in Figure 2.

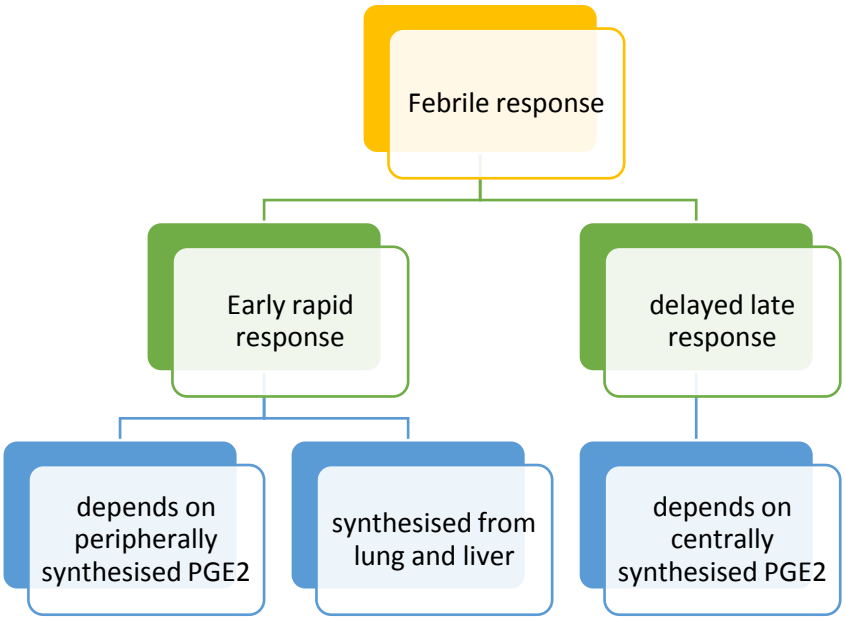


Figure no. 3: Humoral pathway

The second humoral response gets activated directly by the pyrogenic cytokines. The fever signals are directed to the thermoregulatory circuitry by two pathways:

a)direct pathway

b)indirect pathway.

In direct pathway, the circulating pyrogenic cytokines deranges the blood brain barrier. The cytokine or central receptors present in the vascular, glial and neuronal structures of the brain get stimulated and there is release of PGE2 [23]. A de novo synthesis of further release of cytokines by the brain also occurs. In Indirect pathway, pyrogenic cytokines act outside the brain by binding to the cytokine receptors, which leads to release of PGE2 [20,21,23].

THE NEURAL PATHWAY

There is localized formation of PGE2 around the sites of inflammation, where there is activation of cold sensitive cutaneous nerves[31]. Fever signals are transmitted to the areas of the brain responsible for fever generation. Peripheral fever signals reach the central nervous system either through the sensory nerves or by a more complex pathway through the vagus nerve[25-27].

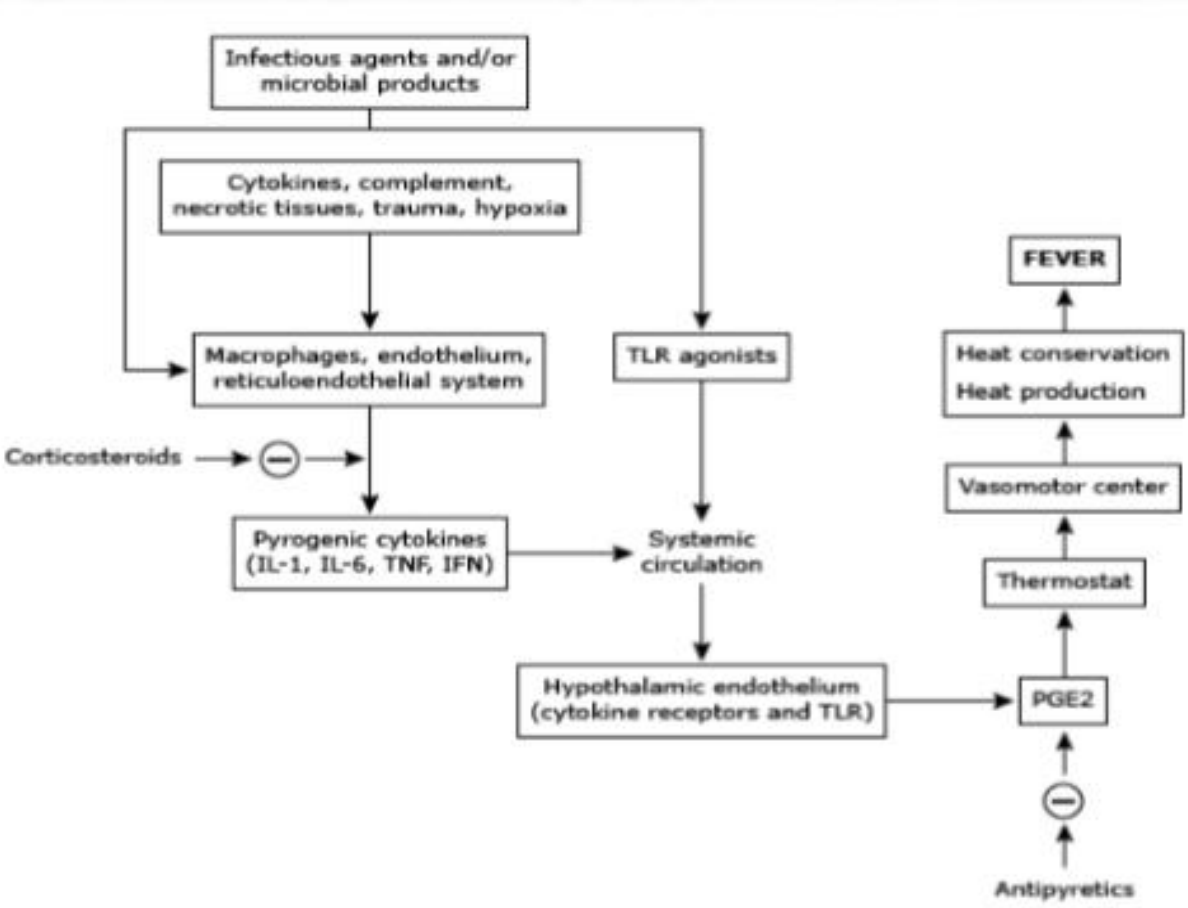


Figure no. 4: Process of fever formation [internet source]

The Kupffer cells of liver is activated by circulating pyrogens which leads to production of endogenous mediators. These mediators activate the hepatic branch of the vagus nerve and the fever signals are produced which are transmitted to the central projection of the area of the vagus nerve within the nucleus of tractus solitarius(NST). From NST, the signals reach the preoptic and hypothalamic areas through the ventral noradrenergic bundle leading to intrapreoptic release of nor epinephrine. A rise in core temperature is evoked by the nor epinephrine through the vagal pathway [25,27].

Etiological causes:

The major diseases groups that are said to cause AUFI are enlisted in Table 2 and the broad classification of AUFI is given in Figure 4.

Table 3 : Major causes of AUFI [19]	
S.No.	**Main disease groups and organisms**
1	Malaria—Including all malaria due to Plasmodium falciparum, P.vivax, P.ovale, P.malariae, P.knowlesi
2	Arboviral infections—Such as dengue, chikungunya, Japanese encephalitis, Zika, yellow fever
3	Enteric fever—Due to Salmonella enterica serovar Typhi and Paratyphi A,B, C
4	Spirochaete infections—Such as leptospirosis and tick-borne or louse-borne relapsing fever
5	Rickettsial infections—Including scrub typhus, murine typhus, spotted fever

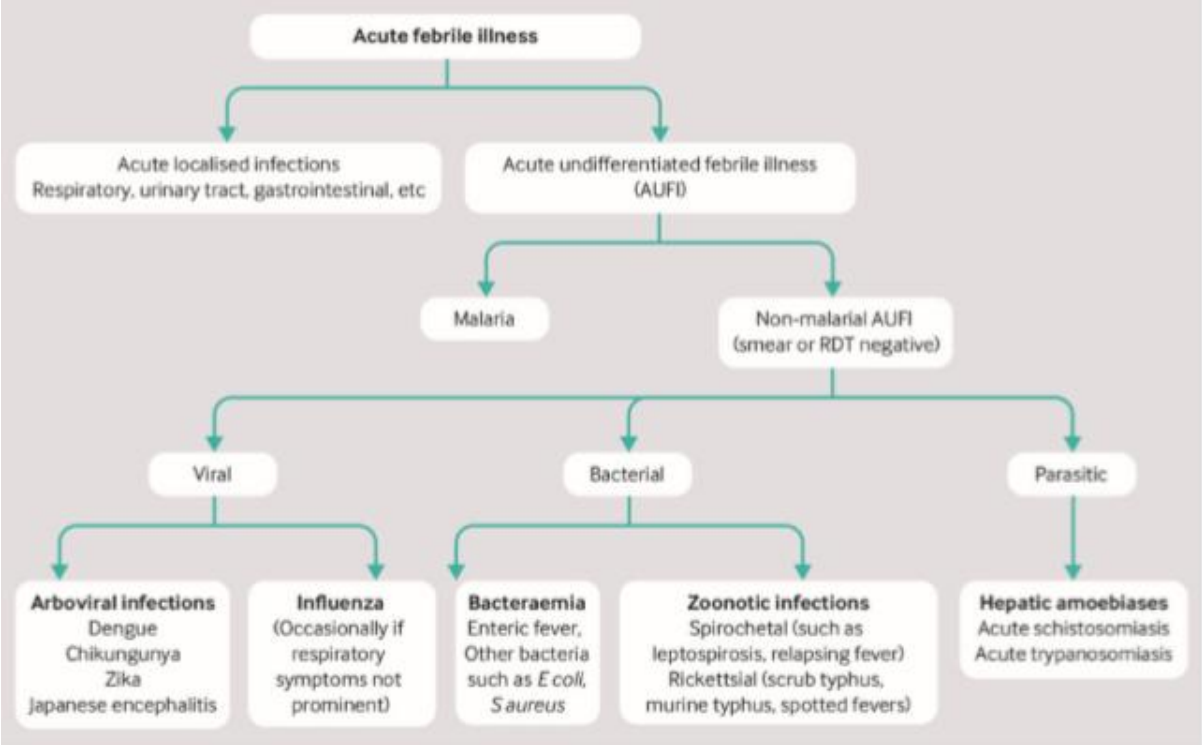


Figure 5: Broad classification of AUFI [19]

Epidemiological aspects of AUFI belonging to major diseases groups in different studies:

Shelke YP et al. [5], conducted a study at Mahatma Gandhi Institute of Medical Sciences, Maharashtra in 2015, to find out the etiological agents in cases of acute undifferentiated febrile illness. The study consisted of adults and pediatric age group cases, who had fever of <2 weeks duration. Patients on antibiotics were excluded. A detailed history, physical examination and baseline investigations were done. Widal test was performed for Typhoid and peripheral blood smear for Malaria. Rapid diagnostic tests(RDT) were used for the diagnosis of Leptospirosis, Dengue, Japanese encephalitis and Scrub typhus. Enzyme-linked immunosorbent assay (ELISA) was done for Leptospirosis, Dengue and Scrub typhus. Tests which turned out negative, were further tested for Dengue.

The most common etiological agent of AUFI in the study was Scrub typhus about 47%, followed by Dengue, about 17.40%. Other common causes were Malaria- 12%, Enteric fever- 4% and Leptospirosis- 2%. The other causes was 10.37% and the undiagnosed AUFI constituted about 6.6%. Male predominance was seen in all the infections except Malaria.

Rani RV et al. [33], had carried a study on identifying the common eitiological agents of acute febrile illness and also their correlation with laboratory parameters at Govt Mohan Kumaramangalam Medical College, Salem, Tamilnadu from December 2015-May 2016. A total of 200 patients were included in the study and blood was collected from them. The serum sample was used for to perform microbiological tests and EDTA blood sample was used for performing quantitative buffy coat and complete blood count analysis.

The commonest etiological agent was Dengue about 27% out of which, 83% were Primary Dengue infection and 17% were secondary dengue, especially around monsoon and post monsoon period. Marked Thrombocytopenia and leucopenia were seen in those cases. Typhoid was seen in 4%, Malaria in 2% and Rickettsial infections in 1%. Leucopenia was markedly seen in typhoid and thrombocytopenia in malaria cases.

Gopalakrishnan S et al.[34],conducted a study from 2010-2012, at A.C.S. Medical College and Hospital, Chennai, to find the etiology along with the clinical markers of AUFI in 403 patients. A detailed history was taken and physical examination performed. Basic laboratory investigations which included Blood counts, Liver function tests Blood basic biochemistry, Urine investigations along with Chest X-ray was performed. Follow up was done till clinical recovery and convalescence. Out of 403 patients,33% were Malaria, 20.59% were Typhoid, 10.4% were Dengue, 6.2% were Leptospirosis. The other causes were 8.9% and unknown causes were 20.84%.

Malaria patients were associated with travel outside the district, jaundice, altered mentation, splenomegaly, elevated AST/ALT levels and thrombocytopenia. Typhoid fever cases were associated with fever of longer duration, abdominal pain and coated tongue. The laboratory findings in those cases were relative bradycardia, low leucocyte count and normal platelet counts. Rash, pruritis, petechiae, retro-orbital pain were the features seen in Dengue fever cases along with low platelet counts. Leptospirosis patients presented with muscle tenderness, conjunctival suffusion, and sub conjunctival hemorrhage. The prevalence of Weil's disease was quite rare.

Chrispal A et al.[13], performed a study at Christian Medical College, Vellore, Tamil Nadu in 2008, to find out the disease spectrum and disease specific profiles of hospitalized AUFI cases in a tertiary care set up. A total of 398 patients were included in the study, a detailed clinical evaluation followed by basic investigations and serological testing was done. The pattern of diagnosis was 47.5% were Scrub typhus, 17.1% were malaria, 8.0% were enteric fever, 7.0% were dengue, 3.0% were leptospirosis, 1.8% were spotted fever rickettsiosis, 0.3% were hanta virus. Alternate diagnosis were seen in 7.3% and unclear diagnosis were seen in 8.0%.

The features associated with scrub typhus were acute respiratory distress syndrome,asepticmeningitis, leucocytosis, hypoalbuminaemia and mild serum transaminase elevation. Malaria was associated with renal failure,splenomegaly, normal leukocyte counts ,moderate to severe thrombocytopenia, hype rbilirubinaemia and mildly elevated serum transaminases. The feature associated with dengue were rash, bleeding manifestations, moderate to severe thrombocytopenia, normal to low

leukocyte counts, and increased hepatic transaminases. Enteric fever presented with loose stools, normal platelet counts and normal to low leukocyte counts.

Phuong HL et al.[35],conducted a study at Binh Thuan province, Southern Vietnam in 2001, to study the characteristics of acute undifferentiated fever in patients who visited the commune healthcare posts and to describe the diagnostic and therapeutic response of the healthcare providers. Two thousand and ninety six patients were included in the study and the gender preponderance, seasonal variation, median age of presentation, presumptive diagnosis and distribution of treatment among these patients were studied.

Male population were mostly affected than females and the median age of presentation was 18 years. The presumptive diagnosis were in the order of pharyngitis, Dengue fever, tonsillitis, typhoid fever, diarrhoea, leptospirosis followed by Hepatitis. The distribution of treatment among patients were self medication, followed by intake of antipyretics along with vitamin supplements and antibiotics.

Singh R et al. [36], conducted a retrospective analysis at tertiary referral centre of Uttarkand in 2013, to study the etiological pattern in acute febrile cases among the population of >12 years of age. The study was carried out for four months and a total of 1141 patients were included. A detailed history and examination were done, slides for malarial parasite and serological tests were carried out. Males were mostly affected due to outdoor activities. Dengue was noted in 71.2%, malaria in 12.8%, typhoid in 8.1% and scrub typhus in 6.0%. Mortality rate was high in dengue cases followed by malaria. Mixed infection were seen mostly in dengue cases and dengue along with malaria were the most common mixed presentation. Mortality rate in mixed infection were 0.09%.

Dengue:

Dengue is widespread throughout the tropics and subtropics [37]. Severe illness is rarely seen (~2% to 4%), although it may be deadly. Most of the worldwide socioeconomic burden of dengue is accountable for less severe cases [38].

Global scenario:

Roughly 1.8 billion (more than 70 percent) of the population at risk of dengue live worldwide in the member states of the WHO South-East Asia Region (SEAR) and the Western Pacific region, with almost 75 percent of today's worldwide dengue-related disease burden. Of SEAR's 11 global places, 10 countries are endemic to dengue along with India[39].

National scenario:

More than a few states / UTs — Andhra Pradesh, Chandigarh, Gujarat, Karnataka, Delhi, Goa, Haryana, Kerala, UttarPradesh, Puducherry, Punjab, Maharashtra, Rajasthan, TamilNadu and West Bengal — recurring outbreaks of dengue fever(DF)/DHF have been reported [40].

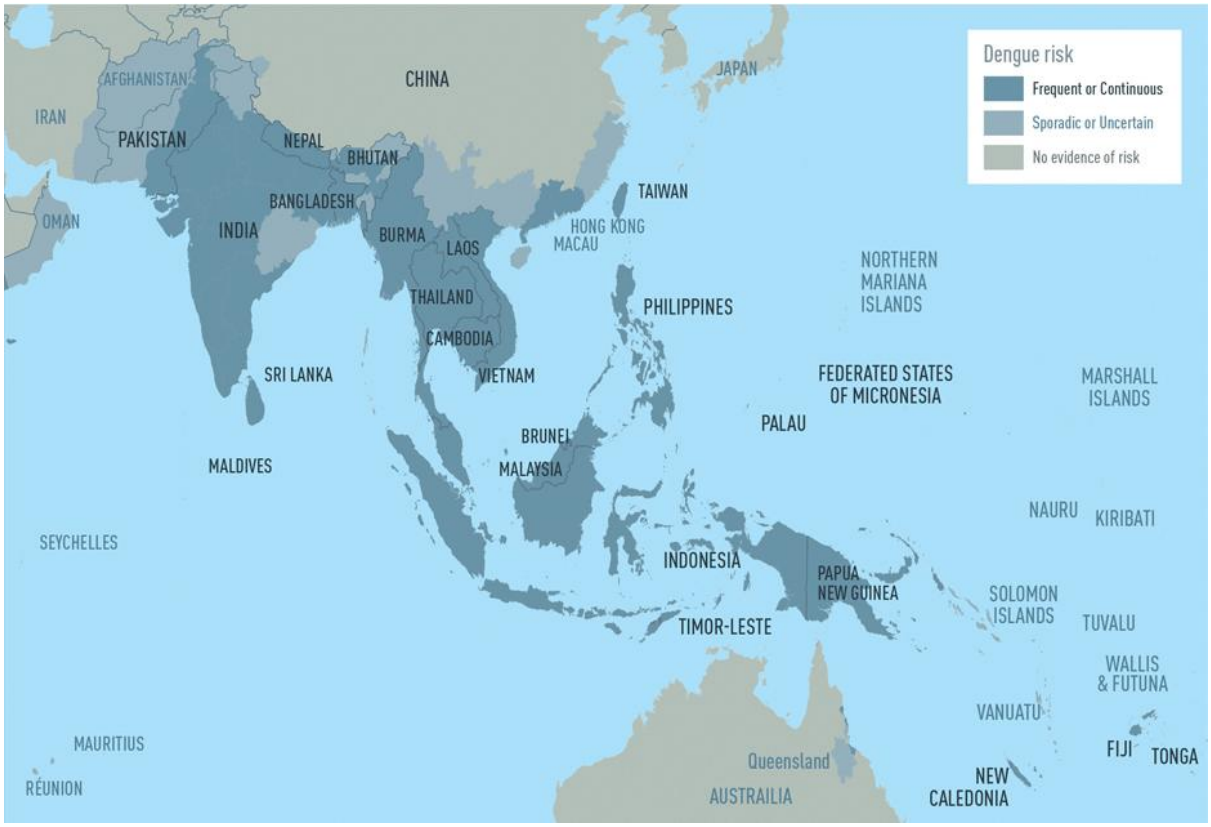


Figure 6 :Global scenario of Dengue among Southeast Asian countries. Source:www.who.int

Epidemiology:

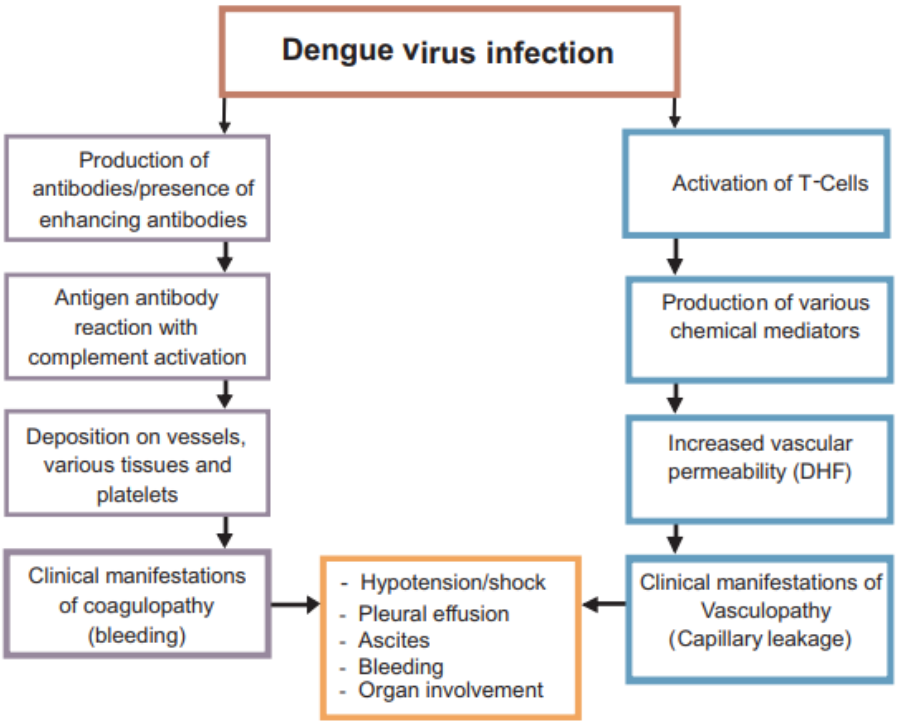


Figure 7: Pathophysiology of DF /DHF(**Source:www.who.int)**

Clinical features:

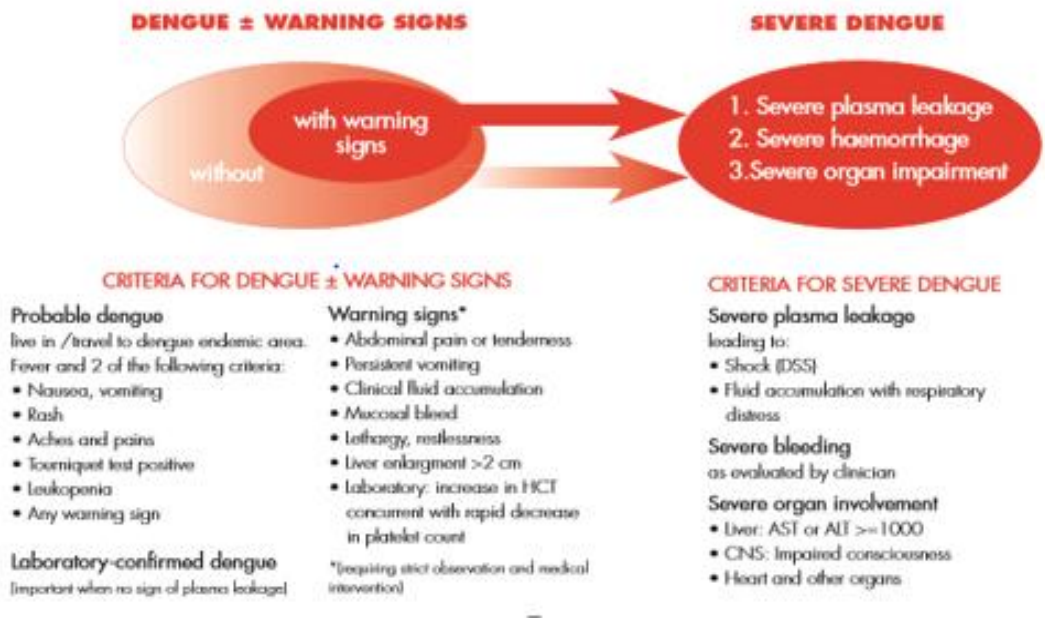


Figure 8: suggested classification of Dengue and the levels of severity [source: WHO dengue guidelines for diagnosis, treatment, prevention and control- new edition 2009]

There were three categories of symptomatic dengue virus diseases: undifferentiated fever, dengue fever (DF) and dengue haemorrhagic fever (DHF). DHF was further categorized into four grades of severity, identified as dengue shock syndrome in grades III and IV [14]. Difficulties in applying the DHF criteria in the clinical situation, along with the increase in cases of clinically severe dengue that did not meet the strict DHF criteria, led to the request for reconsideration of the classification. The DF / DHF / DSS classification is still commonly used today[42,43].

The disease begins abruptly after the incubation period and is followed by the three phases — febrile, critical and recovery [41].

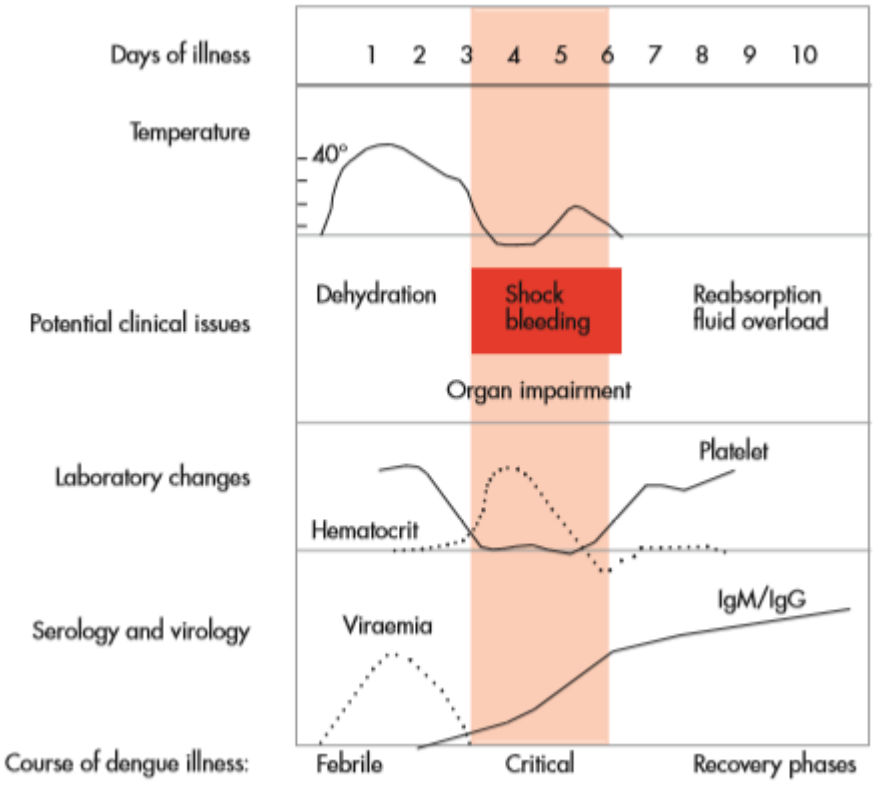


Figure 9: The course of dengue illness [source: WHO dengue guidelines for diagnosis, treatment, prevention and control- new edition 2009]

Laboratory diagnosis:

Rapid Diagnostic Tests:

In recent years, a number of commercial fast format serological test kits for anti-dengue IgM and IgG antibodies have become accessible, some of which

produce outcomes within 15 minutes. Unfortunately, the precision of most of these trials is unsure as they have not yet been correctly validated. Due to cross-reaction with other flavi viruses, malaria parasites, leptospirochetes, and immune illnesses such as rheumatoid and lupus, rapid tests can produce fake beneficial outcomes. In clinical settings, s should not be used to guide the management of DF / DHF instances because many serum samples taken within the first five days of the onset of the disease will not have detectable IgM antibodies. The tests would therefore yield a false negative outcome. In an outbreak scenario, if more than 50 percent of specimens test positive when using fast tests, then it is extremely suggestive that dengue virus is the cause of febrile outbreak [36,44-46]

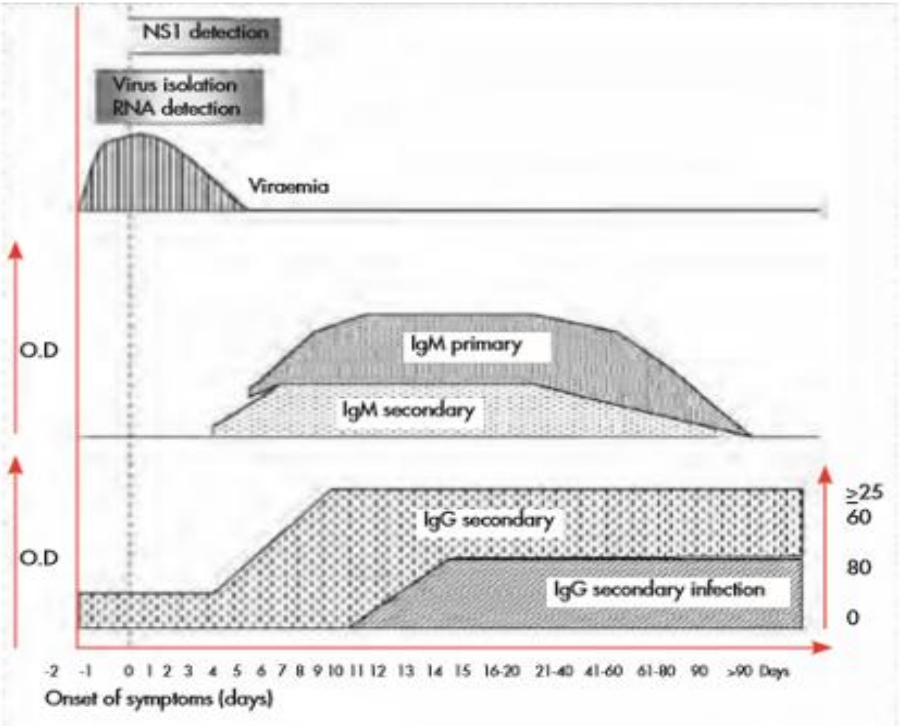


Figure 10: Time- line of primary and secondary dengue virus infections and the diagnostic method used for detection.[source: WHO dengue guidelines for diagnosis, treatment, prevention and control- new edition 2009]

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Serological tests:

Five fundamental serological studies are used to diagnose dengue infection. These are: Haemagglutination-inhibition (HI), complement fixation (CF), neutralization test (NT), IgM capture enzyme-linked immunosorbent assay (MAC-ELISA), and indirect IgG ELISA. For most of these serological trials, the antigen battery should include all four dengue serotypes, another flavivirus, such as Japanese enzymes. Serological confirmation relies on a substantial increase in specific antibodies (four-fold or higher) between acute and convalescent-phase serum samples [36,45]

ELISA based NS1 ANTIGEN TEST:

In both membrane-associated and secretion types, NS1antigen is abundant in the serum of patients during the early phases of DENV infection. As a diagnostic instrument for acute dengue diseases. It's a straightforward, more particular test that demonstrates elevated sensitivity. Due to the specificity of the assay, the NS1 assay may also be helpful for differential diagnosis between flavi viruses. NS1 allows early detection of instances, i.e. in the viremic phase, which has epidemiological importance for transmission containment [36,38]

IgM-capture enzyme-linked immunosorbent assay (MAC-ELISA)

MAC-ELISA uses anti-human IgM to detect dengue-specific IgM antibodies in the test serum. The IgM antibody anti-dengue grows slightly quicker

than IgG and is generally detectable by day 5 of the disease. Some patients on days 2 to 4 after the onset of disease have detectable IgM, while others may not develop IgM for seven to eight days after the start. In some primary infections, detectable IgM may persist for more than 90 days, but in most patients it wanes to an undetectable level by 60 days. In regions where dengue is not endemic, it can be used for viral disease or random population-based sero surveys in clinical surveillance, with the assurance that any positive detected are latest infections. It is particularly helpful for patients in hospital who are usually admitted late in the disease after detectable IgM is already present in the blood [36,37,39].

Isolation of dengue virus:

The sample is drawn and processed without delay in the first five days of disease. Specimens that may be appropriate for virus isolation include the patient's acute phase serum, plasma or washed buffy coat, autopsy tissue from deadly instances, particularly liver, spleen, lymph nodes, and naturally gathered thymus and mosquitoes. Virus isolation takes 7–10 days, so starting patient management with DF / DHF may not be very useful [38,39].

Polymerase chain reaction (PCR):

Molecular diagnosis based on inverse transcription polymerase chain response (RT-PCR) such as single-stage or nested RT-PCR or RT-PCR in real time has gradually superseded the virus isolation technique as a new standard for dengue virus identification in acute serum samples [44].

Isothermal amplification method:

Nucleic acid sequence-based amplification (NASBA) is an isothermal RNA-specific amplification test that does not require instrumentation for thermal cycling. The RT-PCR methods ' sensitivity ranges from 80% to 100%. High sensitivity and specificity, ease of identification of serotypes and early detection of infection are the benefits of this technology. However, it is an costly technology requiring advanced instrumentation and qualified workforce [36,44-46]. Recently, the PCR technique of Loop Mediated Amplification (LAMP) has been created [36,39].

Indications	Diagnostic Tests	Advantages	Limitations
Diagnosis of acute dengue infection	Nucleic acid detection	•Most sensitive and specific •Possible to identify serotype •Early appearance (pre-antibody), so opportunity to impact on patient management	•Potential false positive due to contamination •Expensive •Needs expertise and expensive laboratory equipment •Not possible to differentiate between primary and secondary infection
	Isolation in cell culture and identification using immuno-fluorescence	•Specific •Possible to identify serotype by using specific antibodies	•Need expertise and facility for cell culture and fluorescent microscopy •Takes more than 1 week •Not possible to differentiate between primary and secondary infection
	Antigen detection in clinical specimens	•Easy to perform •Opportunity for early diagnosis may impact on patient treatment	•Not as sensitive as virus isolation or RNA detection
	Serologic tests: IgM tests Seroconversion: 4-fold rise in HI or ELISA IgG titres between acute and convalescent samples	•Useful for confirmation of acute infection •Least expensive •Easy to perform •Can distinguish between primary and secondary infection	•May miss cases because IgM levels may be low or undetectable in some secondary infections •Need two samples •Delay in confirming diagnosis
Surveillance and outbreak identification; Monitor effectiveness of interventions	IgM detection	•Identify probable dengue cases •Easy to perform for case detection in sentinel laboratories	•May miss cases because IgM levels may be low in secondary infections
	Viral isolation and RNA detection	•Confirm cases •Identify serotypes	•Can be performed only in reference laboratories •Need acute samples

Table 4:Various dengue diagnostic tests – Advantages and Interpretations[source: WHO dengue guidelines for diagnosis, treatment, prevention and control- new edition 2009]

Typhoid:

Enteric fever is an acute, febrile illness, which is life-threatening and caused by members of certain Salmonella serotypes: Salmonella typhi, Salmonella paratyphi A, and S. paratyphi B. [55,56]. Annually about 11–21 million cases and 1,28,000 –1,61,000 deaths occur in typhoid fever and in paratyphoid fever, approximately 6 million cases and 54 000 deaths are affected annually [48, 51-53].

The case fatality rate of typhoid fever was about 10–30% in patients without treatment and with appropriate therapy, the rate dropped down to 1–4%[54].

Clinical features:

The disease can present in three forms:

- Acute non complicated disease
- Complicated disease
- Carrier state

Acute non-complicated disease: Symptoms of prolonged fever, headache, malaise and anorexia, Bronchitic cough, disturbances of bowel function (diarrhoea in children, constipation in adults) are seen. 25% of patients develop exanthem (rose spots), on the chest, back and abdomen.

Complicated disease: Upto 10% of population develop complcations. The common signs observed are presence of occult blood (10-20%), melena (3%) in pathologies of gut-associated lymphoid tissue. Intestinal perforation and peritonitis is reported in 3% of hospitalized cases. The symptoms associated are diffuse abdominal discomfort confined to the right lower quadrant. The signs include hypotension, sudden rise in pulse rate, marked abdominal tenderness along with rigidity, rebound tenderness and guarding.

Other complications seen along with typhoid fever include: thrombocytopenia, hepatitis, myocarditis, haemolytic uraemic syndrome, pneumonia, disseminated intravascular coagulation and haemorrhages.

Carrier state: S.typhi is harboured in the gallbladder in 1- 5% of patients [49]

Laboratory diagnosis:

The isolation of S.typhi from blood, bone marrow, urine, feces and other fluids is required for the definitive diagnosis of the disease [50]. The preferred sample for the diagnosis of enteric fever and invasive non typhoidal Salmonella infections especially in endemic setting is Blood [55,56]. Still, the gold standard for the diagnosis of typhoid fever is Bone marrow aspirate culture [57-59], particularly in

patients who have been already treated, long history of illness and also for patients showing negative blood culture [60].

Widal test is an oldest, presumptive serological test used for diagnosing Enteric fever. It works on the principle of tube agglutination test. The concept was given by Georges Fernand Isidore Widal, in the 18th Century, Algeria. This test is detects the presence of O and H antibodies in the patient's sera by using O and H antigens. The antigens are prepared using *S.typhi* 901 'O' and 'H' strains and S.paratyphi A and B (Laboratory strains).

Significant titre (all over India) is taken as : >200 for H agglutinin and >100 for O agglutinin. False positive can be seen in anamnestic response, Inapparent infections, prior immunization. This can be confirmed by demonstrating a fourfold rise in titre in paired sera, taken a week apart. Also the titre falls down in a week in anamnestic response, while there is a fourfold rise after a week in true infection. False negative can occur when blood is drawn in the first week or fourth week, carriers, and following antimicrobial therapy. Despite constraints, Widal is still used for diagnostic purposes all over the world. [61,62]

Recent studies show that serological tests have poor sensitivity and inadequate specificity, and are inappropriate for use in routine purposes, yet serologic tests continue to be commonly used in many settings [63]. Due to these reasons, Confirmation by culture is essential for non-specific febrile illness such as typhoid fever, paratyphoid fever and other invasive salmonellosis, specially when only a single sample is collected and history of extensive ingestion of antimicrobials before hospitalization [64].

Malaria:

Malaria is a significant health problem in Sub-Saharan Africa and South Asia, including India, in tropical and developing nations, particularly in infants and pregnant females. Billions of these areas face malaria danger each season and more than one million die each year. Although all four Plasmodium species can cause

malaria in humans, Falciparum malaria is the most deadly if not treated [65]. It is unusual to infect more than one Plasmodium species at the same time.

In India, malaria is most contributed by the state of Odysa, contributing 25 percent of a total of 1.5–2 million recorded annual cases of malaria, 39.5 percent of P. falciparum malaria, and 30 percent of Indian malaria-related fatalities. Twelve states, namely Odisha, Gujarat, Jharkhand, Maharashtra, Chhattisgarh, Madhya Pradesh, Uttar Pradesh, Assam, West Bengal, Rajasthan, Haryana and Andhra Pradesh, recorded 90 percent of instances of malaria [66].

Fever is the cardinal symptom of malaria that, accompanied by chills and rigors, can be intermittent with or without periodicity. Headache, nausea, vomiting, myalgia, anorexia and arthralgia are specific symptoms. Non-specific symptoms can imitate other illnesses such as viral infections, enteric fever, etc. Microscopy and/or rapid diagnostic test (RDT) should investigate all clinically suspected instances of malaria immediately [66,67].

The National Vector Borne Disease Control Program (NVBDCP) prescribes that malaria's annual blood test rate (ABER) should be at least 10 percent on the assumption that at one stage in time 10 percent of the population will have fever in a year [68].

The evolving clinical manifestations with multi-organ participation in P. falciparum, emerging trends in P.vivax malaria complications, and the burden of pregnancy malaria are other significant problems that merit attention and formulation of appropriate intervention strategies [69].

Leptospirosis:

Leptospires are Obligate aerobic, gram-negative, flexible, motile helical rods spirochete bacteria in the genus *Leptospira* [70]. They are too thin to be seen under the light microscope and are best viewed under the dark ground microscopy. They cannot be easily stained with aniline coloring and can be stained by Giemsa stain only slightly. Leptospires are best colored by techniques of silver impregnation [70-72]

Transmission:

The path of infection is through skin abrasions or cuts, or through the membranes of the conjunctive and mucous membranes [70]. People may be infected from infected animals by direct contact with urine or by reproductive fluids. Indirect infection can happen when contaminated water or moist soil is in contact or when contaminated food or water is consumed. Infection rarely happens by bites of animals or contact between human [73].

Epidemiology:

Leptospirosis is distributed globally; the incidence in tropical environments is greater, and the estimated annual incidence globally is > 1 million. South and Southeast Asia, Oceania, the Caribbean, parts of sub-Saharan Africa, and parts of Latin America are projected to be the largest morbidity regions [74]. Outbreaks may happen in endemic regions after heavy rainfall or flooding, particularly in urban regions of developing nations where housing and sanitation circumstances are poor. Leptospirosis has been a significant cant issue in India's low-lying regions that are densely populated and susceptible to fluorescence and water stagnation during monsoon. Leptospirosis outbreaks from Kerala, Gujarat, Tamil Nadu and Karnataka are increasingly recorded. Cases from Goa, Andhra Pradesh and Assam were recorded [77]. Travelers taking part in recreational freshwater operations, such as swimming or boating, are at enhanced danger, especially after heavy rainfall or flooding and after extended immersion in contaminated water or swallowing [74-76].

Clinical Presentation:

The duration of incubation is 2-30 days, and disease generally happens 5-14 days after exposure. While most infections are assumed to be asymptomatic, clinical disease may happen as a self-limiting acute febrile disease, estimated to happen in about 90% of clinical diseases, or as a serious, possibly deadly, multi-organ dysfunctional disease in 5%-10% of patients [70-72]. The disease may be biphasic in patients progressing to serious disease, with a temporary reduction in fever between stages. The acute septicemic stage (roughly 7 days) presents as an acute febrile disease with symptoms including headache (can be serious and include retro orbital pain and photo-phobia), chills, myalgia (characteristically involving the calves and lower back),

conjunctival suffusion (characteristics of leptospirosis but not happening in all instances), nausea, vomiting, diarrhea, abdominal pain, cough, etc. Antibody manufacturing and the existence of leptospires in the urine characterize the second or immune stage. Symptoms may include jaundice, renal failure, hemorrhage, aseptic meningitis, cardiac arrhythmias in patients with serious disease, pulmonary insufficiency, and hemodynamic collapse. Weil illness, the classically described syndrome, comprises of kidney and liver failure and has a case-fatality ratio of 5%–15%. Severe hemorrhagic pulmonary syndrome is a rare but serious type of leptospirosis with a case-fatality ratio of > 50 percent. Poor prognostic indicators include elderly age and modified mental status, respiratory failure or oliguria [72,74-76].

Diagnosis:

Leptospirosis diagnosis is generally based on serology; the gold standard is microscopic agglutination test and can only be conducted in some reference laboratories [73]. Culture is insensitive, but detection of the organism in blood or cerebrospinal fluid (for meningitis patients) using PCR in real time may provide a more timely diagnosis during the septicemic stage. There are several serological screening tests available, including ELISA and various fast diagnostic tests; with the microscopic agglutination test, favorable screening results should be verified. Leptospirosis is a disease that can be reported at national level [74,76,78].

Treatment:

Early antimicrobial therapy may be efficient in reducing the seriousness and frequency of leptospirosis and should be launched early, if leptospirosis is suspected, without waiting for confirmatory test outcomes. Doxycycline is a choice medicine (100 mg orally, twice daily) for patients with mild symptoms but is not recommended for pregnant females or infants < 8 years of age; alternatives include ampicillin and amoxicillin. For patients with serious leptospirosis, intravenous penicillin (1.5 MU every 6 hours) is a drug of choice and ceftriaxone has been shown to be similarly efficient. Patients with serious leptospirosis may need hospitalization and supportive therapy, including intravenous hydration and supplementation with

electrolytes, oliguric renal failure dialysis, and respiratory failure mechanical ventilation [80].

Prevention:

There is currently no available vaccine. Travelers at enhanced danger of infection should be trained on exposure hazards and recommended to consider preventive interventions such as chemoprophylaxis; to wear protective garments, particularly footwear; and to cover cuts and abrasions with occlusive dressings [71,72]. Limited trials have shown that doxycycline chemoprophylaxis (200 mg orally, weekly), started 1–2 days before and continued through the exposure period, may be efficient in the prevention of clinical illness in adolescents and may be regarded for individuals at high risk and with short-term exposure. Avoiding exposure is the best way to prevent infection: Travellers should avoid contact and walking in flood waters, and contact with possibly infected livestock or their secretions [78-81].

Scrub typhus:

Scrub typhus, caused by Orientia tsutsugamushi (formerly Rickettsia), is an acute variable severity infectious disease transmitted to humans by a Trombiculidae family arthropod vector. Due to the sort of vegetation that harbors the vector, the term "scrub" is used. The word "typhus" is derived from the Greek word "typhus," meaning "stupor fever" or smoke [82].

It impacts individuals, including young children, of all ages. In this zoonotic disease, humans are accidental hosts. While scrub typhus is geographically restricted to the Asia Pacific area, one billion individuals are at risk and almost one million instances are recorded annually [83].

Overall mortality ranged from 7% to 9%, mortality levels in untreated patients ranged from 0% to 30% and appeared to vary with the era and area of infection of patients. In India, during the Second World War, scrub typhus broke out in an epidemic form in Assam and West Bengal. Subsequently, this disease was discovered in humans, Trombiculid mites and rodents throughout India [84]. Mortality

in many fields is still around 15 percent owing to missed or delayed diagnosis [85]. Mortality is greater when serious complications such as ARDS occur.

Pathogen:

Orientia tsutsugamushi is an obligate, Gram-negative, intracellular bacterium with many serotypes. It involves heterogeneous strains categorized into five main serotypes: (1) Boryon, (2) Gilliam, (3) Karp, (4) Kato and (5) Kawazaki [86]. There are several genetic variations in each geographic region and these vary from genetic variants in other areas.

Epidemiology:

Some species of trombiculid mites ("chiggers," Leptotrombidium deliense and others) transmit scrub typhus to humans and rodents. People get the disease from an infected chigger's bite [87]. The mite's bite leaves a distinctive black Eschar. The lifecycle of the adult mites is four-stage: (1) egg, (2) larva, (3) nymph and (4) adult. The larva is the only phase (chigger) capable of transmitting the disease to people and other vertebrate people.

Chigger mites function as O.tsutsugamushi's main reservoir. Scrub typhus is endemic to a portion of the globe known as the 'Tsutsugamushi triangle,' which stretches from southern Japan and far-eastern Russia in the north to southern Australia in the south and Pakistan in the west [88]. Some Asian nations have recorded an increase in the incidence of scrub typhus, which coincides with the extensive use of b-lactam antimicrobial medicines and urbanization. It is a re-emerging infectious disease in India [89,90]. Outbreaks have occurred in regions from Jammu to Nagaland situated in the sub-Himalayan belt. There have been reports of outbreaks of scrub typhus in Himachal Pradesh, Pondicherry, Sikkim, Darjeeling and Tamil Nadu [91-93].

It occurs more frequently during the rainy season. However, outbreaks have been reported during the cooler season in southern India [94]. Scrub typhus is difficult to recognize because the symptoms and signs are often nonspecific. The nonspecific presentation and lack of the unique Eschar results in misdiagnosis and under-

reporting in 40–60 percent of patients. On the other hand, diagnostic facilities are not available anywhere. The accurate incidence of the disease is therefore unknown. In untreated patients, mortality rates range from 0–30% [91].

Clinical features:

The scrub typhus clinical spectrum is wide, with most diseases of mild to moderate severity. The first sign of illness in patients after an incubation period of 7–21 days (mean 10–12 days) is a vesicular lesion at the mite feeding site, which later becomes an eschar or a regional lymphadenopathic ulcer. At the chigger bite site, an eschar is seen and is frequently observed in the groin, axilla, genitalia and neck. It is the most significant single clue for diagnosis and when seen by an experienced physician and it is pathognomonic. Several eschars can rarely be noticed [95].Severe headache happens almost invariably and is a main criterion for identifying suspected instances.

Specific serological tests:

The Weil Felix test is the cheapest and easiest serological test available. During the second week, 50% of patients have a favorable test outcome. This test, however, lacks specificity and sensitivity [96,97]. Indirect immunofluorescence antibody (IFA) is the gold standard. Indirect immunoperoxidase (IIP) is a modification of the conventional IFA technique that can be used with a light microscope and the findings of such trials are similar to those of IFA [98]. Serological techniques are most accurate when looking for a four-fold increase in antibody titer.

Qualitative immunosorbent enzyme-linked assay (ELISA) for immunoglobulin M (IgM) antibodies to O. Serum tsutsugamushi is now accessible commercially. This is using an O. Recombinant antigen combination obtained from tsutsugamushi. This test helps to diagnose human exposure to the species O.tsutsugamushi.

The organism can be cultivated from the blood of scrub typhus patients in tissue culture or cattle. O.tsutsugamushi isolation needs Biosafety level-3 equipment and it takes 27 days for the average moment to be positive.

Samples such as Skin rash biopsies, lymph node biopsies, or ethylenediaminetetraactic acid (EDTA) blood can be used in Polymerase chain reaction (PCR). O.tsutsugamushi can be proved by standard and nested PCR [98]. Real-time PCR assays are as delicate as conventional PCR assays but are faster and can yield quantitative results [99].

Prevention:

No efficient scrub typhus vaccines are available. O.tsutsugamushi strains have a huge antigenic variation and immunity to one strain does not confer immunity to another strain. To provide an appropriate amount of safety, any scrub typhus vaccine should provide protection for all strains current locally. This complexity is still hindering attempts to create a feasible vaccine [99].

4.0. Materials and methods:

A Hospital based Prospective Cross sectional study was carried out at the Department of Microbiology, Trichy SRM Medical College Hospital and Research Centre, Irungalur, Trichy, Tamilnadu, for a period of one year (January 2018 to December 2018), after an approval from the Institutional research board and Institutional ethics committee (copy enclosed – Annexure –I). Informed consent was obtained from every participant in vernacular language.(model copy of informed consent enclosed – Annexure-II).

4.1. Materials:

Inclusion criteria:

Consecutive cases of both sexes and all adults(age more than 15 years) admitted to medical wards with a history of fever less than 10 days and continued to have oral temperature above 37.5^0 c were considered for the present study.

Exclusion criteria:

1. Patients on antimicrobial drugs
2. Patients who are Immunocompromised or on immunosuppressants.
3. Those who have any one of the following or a combination of them namely, focal sepsis, skin and soft tissue infections, urinary tract infections, infective endocarditis, osteal and hematological disorders, respiratory tract infections, who are on devices, recent surgeries and surgical site infecions, autoimmune disorders, overt malignances, traumatic injury or vascular accidents.

4. Pregnant women or those <15 years were not included.
5. Semiconscious, conscious or comotosed cases.

4.2. Patient history:

After enrollment in the study a detailed history was taken, which included age, sex, demographic details, history of presenting illness, past illness, occupation, , history of vaccination, animal bite/handling, visit to foreign countries, transplantations, immune suppressive drugs, history of co-morbid conditions, medication history(current and past), personal history like substance abuse, smoking, alcohol intake, etc.

Detailed clinical history such as duration of fever, headache, chills, cough, dyspnoea, myalgia, nausea and vomiting, calf tenderness, dyspnoea, diarrhoea/dysentry, jaundice, abdominal pain, rash/eschar, arthralgia, altered mentation, bleeding manifestation, retro orbital pain, burning micturition, focal sepsis, skin and soft tissue infections were elicited. Following this, patients were subjected for a detailed clinical examination of various systems. The parameters were fever, heart rate, respiratory rate, blood pressure, erythrocyte sedimentation rate, eschar, pedal edema, cyanosis, hepatomegaly, splenomegaly, lymphadenopathy, petechiae and ALOC. Following clinical examination, all patients were subjected to the investigations based on duration of fever.

The investigations were Complete blood count, peripheral smear study, urine albumin, blood urea, serum creatinine, liver enzymes and serum bilirubin,

coagulation profile X-ray chest and diabetic screening were carried out if required. Depending upon detailed examination on suspicion of tropical infections MP smear, Dengue NS1 antigen and IgM antibody, Widal test, Scrub typhus IgM , Leptospirosis IgM, Chikungunya IgG/M were performed along with blood culture for Enteric fever group of bacteria and urine culture.

Patients in the study group were followed up till outcome for complications like shock, bleeding manifestations, hepatic failure, renal failure, respiratory distress, cardiac failure, pulmonary edema, musculo skeletal complications and multi organ failure. Final diagnosis was arrived in these patients based on clinical and / or laboratory parameters for the selected group of AUFI. Patients with AUFI's but could not be placed in any of the common diagnosis either clinically or by investigations were labeled as undiagnosed fever for this study category and not included for the study purpose. Outcome was defined as recovery before hospital discharge, death or Against Medical Advice.

Data were entered in Excel Spreadsheet and analyzed using SPSS software Version 22. Simple calculations like Percentages, Proportions and Mean values were derived. Appropriate statistical tests like Chi- Square test, T test were used to compare the various study parameters among the patients. Data was analyzed for any statistical significance with a P value < 0.05 being considered significant.

4.3. Sample collection

A total volume of 15 ml of blood was collected from each patient, one 5ml of blood sample was inoculated into blood culture bottles (Brain Heart Infusion

Broth) and transported to the laboratory within 2 hours. The second 5 ml of blood was collected in plain tube for serological tests and biochemical tests and the third 5ml in ethylene diamine tetra acetic acid (EDTA) bulb for hematological profile. Peripheral smear -Thick and Thin smear was prepared for malaria microscopy from the last few drops of blood. Blood collected in the plain bulb was centrifuged at 3000 g for 10 min, and serum was stored at minus 20°C for serological testing. Serum was used for Widal test as well as for other serological tests for the diagnosis of malaria, scrub typhus and chikungunya by rapid diagnostic tests (RDTs) and Enzyme-linked immunosorbent assay (ELISA) for dengue and leptospirosis. Heterophile agglutination test (Weil-Felix test) was done for rickettsial diseases. EDTA blood sample was used for Quantitative Buffy Coat analysis and complete blood count analysis.

Diagnostic criteria: [11]

- Dengue: Clinical features of dengue with dengue specificNS1 antigen/ IgM antibody positive and other serological tests and blood culture negative or seroconversion on convalescent sera.
- Malaria: Malarial parasite (trophozoites of Plasmodium falciparum, Plasmodium vivax or mixed) visualized on thin blood smears.
- Enteric fever: Blood culture positive for Salmonella typhi or Salmonella paratyphi or 4-fold rise in titer in the Widal test in paired sera, when blood culture was negative.
- Scrub typhus: Eschar + scrub IgM positive by Immunochromatographic test or Scrub IgM ELISA positive with other serological tests and blood culture negative or scrub IgM ELISA seroconversion on convalescent sera

- Leptospirosis: Leptospira specific IgM antibody positive with other serological tests and blood culture negative.
- Unclear diagnosis: After complete evaluation, a definitive diagnosis was not made. This included patients who did not have convalescent sera taken (diagnostic criteria were not fulfilled during initial admission) and who had multiple serological tests positive without fulfilling the diagnostic criteria.

4.4. Laboratory work (Methodology)

4.4.1. Peripheral smear preparation for malarial parasite:

The thin and thick blood smears were prepared from the E.D.T.A blood. A clean, grease free slide was taken and a drop of blood was placed on one side about 1-2 cms from the end. A spreader slide was placed at an angle of 45° in front of the drop and it was moved back so as to make contact with the drop followed by a rapid smooth forward movement. A film of about 3-4 cm in length was made in the shape of tongue, which occupied about 2/3rd of the slide. The slide was then air-dried and fixed using methanol. The thick blood film was prepared by placing three drops of blood on the corner of the slide and the blood was spread in a circular form and made to a diameter of 1-2 cms. The slide was allowed to air dry and dehemoglobinization was done by immersing the thick smear in distilled water for about1-2 minutes. Both the thin and thick blood films were stained using Jaswant Singh-Bhattacharya stain and viewed under the microsope with oil immersion (100 x) objective. The smear was re-examined by a senior assistant professor and guide.

4.4.2. Dark field microscopy for leptospira:

Blood:

The Blood sample (with anticoagulant- Heparin) was centrifuged for 15 minutes at 1000 g and a volume of 10 µl of plasma was taken on a microscopic slide and the cover slip was placed over the sample and was examined using Dark field microscopy under low and high power (×10 and ×40). If no leptospira was seen, the plasma was further centrifuged for 20 minutes at 3000–4000 g and a drop of sediment was placed on a slide with a cover slip and observed. Determination of the number of Leptospira seen is done by simple counting and the result was taken as Leptospira positive per high power field or per 100 high power fields (varies upon the concentration). The result was taken as negative for Leptospira, if no Leptospira was observed in 100 high power fields when done using high speed centrifugation. The smear re-examined by a senior assistant professor and guide.

Urine:

A volume of 3 ml of freshly voided urine was centrifuged for 10 minutes at 3000 g. The supernatant was discarded and the deposit was taken on a microscopic slide and the cover slip was placed over the sample and was examined using Dark field microscopy under low and high power (×10 and ×40). The smear re-examined by a senior assistant professor and guide.

4.4.3. Blood culture:

Blood culture is the ideal method for diagnosing Enteric fever especially during the first week of illness. The procedure was carried out by collecting blood of volume 5 ml in a blood culture bottle containing 25 ml of brain heart infusion broth.

The dilution made was 1:5. The bottles were incubated at 37°C and growth was observed from 24 hours upto 7 days. The subcultures were carried out everyday for 1 week onto Nutrient agar, 5% Sheep blood agar and Mac Conkey agar plates by adopting standard microbiological techniques. The plates were incubated in an aerobic environment for 24 hours and then plates were read and the isolates were identified based on colony morphology, Gram stain, motility and biochemical tests. The tests used for identification of gram negative bacilli include colony morphology, Gram's smear, preliminary tests like catalase test, oxidase test followed by hanging drop for detection of motility, production of indole and urease, utilization of citrate, fermentation of Triple sugar iron agar, Methyl red test, Voges Proskauer test and nitrate reduction test were also performed. Antibiogram was performed for the Enteric group of bacteris if identified using Kirby-Bauer disk diffusion method as per the CLSI 2018 guidelines.

4.4.4 Serological tests:

All the serological tests were carried out as per the manufacturer's instructions given in kit insert. The results were seen by Senior Assisstant Professor and guide. If any controversy was there, the matter was settled by Professor.

WIDAL TEST:

Principle:

The antibodies to O and H agglutinins in the sera react and agglutinate serial doubling dilutions of killed coloured Salmonella antigens in a slide and tube agglutination test.

Slide agglutination test:

Table no.5	
S.no	**Procedure**
1	One drop of positive control and Isotonic saline was pipetted on first and second reaction circles of the slide (positive and negative Control).
2	One drop of the patient's serum was pipetted onto the remaining four reaction circles.
3	A drop of Widal suspension 'H' antigen was added to the first two reaction circles.
4	A drop of each of 'O', 'H', 'AH' and 'BH' antigens were added to the remaining four reaction circles.
5	The contents were mixed uniformly with separate mixing sticks
6	The slide was rocked back and forth and observed for agglutination, within one minute.

Interpretation:

1. positive test result- a clear visible agglutination.
2. negative test result - no agglutination

Tube agglutination test: Requirements:

1. Test tube rack
2. Round-bottomed Felix tubes
3. Conical-bottomed Dreyer's tubes
4. Water bath

5. Killed coloured suspensions of *S.typhi* O antigen, *S.typhi* H antigen, *S.paratyphi* AH antigen, *S.paratyphi* BH antigen.
6. Incubator

Procedure:

Table no.6

S.no	**Procedure**
1	Three sets of 8 test tubes were taken in a widal rack for S.typhi O, S.paratyphi AH, S.paratyphi BH and they were labelled as no.1 to 8. A set of 8 Dreyer's tubes for S.typhi H antibodies were kept in a separate rack The last test tubes in the four rows were taken as the saline/negative control (0.5ml of 0.85% of saline).
2	1.9 ml of physiological saline was taken in the tube no.1 of the three rows, to which 0.1 ml of the patient's serum was added and mixed well to prepare Master dilution.
3	A volume of 1.0 ml of physiological saline was added from tube no.1 to tube no.8.
4	1.0 ml of diluted serum was transferred from tube no.1 to tube no.2 and mixed well
5	The sample was serially diluted in the similar fashion from tube no.2 to tube no.7 in all the four rows.
6	1.0 ml of diluted serum was discarded from tube no.7 of each set. The dilution achieved in each set of the serum sample was:

	<table><tr><td>Tube no.</td><td>1</td><td>2</td><td>3</td><td>4</td><td>5</td><td>6</td><td>7</td></tr><tr><td>Dilution</td><td>1:20</td><td>1:40</td><td>1:80</td><td>1:160</td><td>1:320</td><td>1:640</td><td>1:1280</td></tr></table>
7	50 µl of respective antigen is added to the corresponding row i.e. first row with Salmonella typhi 'O' antigen, second row with Salmonella paratyphi 'AH' antigen, third row with Salmonella paratyphi 'BH' antigen and S.typhi ‘H’ antigen kept in a separate rack.
8	The tubes were mixed well and incubated overnight at 37°C.

Observation:

1. Positive test result:
    - H antibodies- large loose fluffy cotton-woolly clumps with clear supernatant fluid.
    - O antibodies – compact granular chalky clumps with clear supernatant fluid.
2. Negative test result: button formation (no agglutination).

Dengue NS1 antigen by MICROLISA (J. Mitra)

Principle:

Dengue NS1 antigen MICROLISA is a solid phase enzyme linked immunosorbent assay based on the principle of “Direct sandwich”. A sandwich complex is formed between the dengue NS1 antigen and antibody horseradish peroxidase, where the Dengue NS1 antigen is trapped in between them. The substrate buffer and chromogen is added, upon which blue colour is developed. Stop solution is

added to limit the enzyme-substrate reaction and the developed yellow colour was read with the help of 450nm spectrophotometrically [100].

Components of the kit:

Table no.7 Components of the kit [100]		
S.no.	**Component**	**Description**
1	Microwells	Coated with anti-Dengue NS1 antibodies.
2	Diluent (sample and conjugate dilution)	Buffer containing protein stabilizers and antimicrobial agents.
3	Enzyme conjugate concentrate (50x)	Monoclonal Anti-Dengue NS1 linked to horseradish peroxidase with protein stabilizers.
4	Wash buffer concentrate(25x)	Concentrated phosphate buffer with surfactant.
5	TMB substrate	TMB, to be diluted with TMB diluent before use.
6	TMB diluent	Buffer solution containing hydrogen peroxide with preservatives.
7	Positive control	Recombinant NS1 antigen with preservative.
8	Negative control	Normal human serum negative for Dengue NS1 antigen with preservative.
9	Calibrator	Recombinant NS1 antigen with preservative.
10	Stop solution	Ready to use, 1N Sulphuric acid.

Procedure:

Table no.8[100]

S.no.	Procedure	Reagents
1	Add controls, calibrator and samples.	50 µl of diluent, 50 µl of controls, calibrator and samples.
2	Prepare working conjugate	No of strips: 1, 2, 3, 4, 5 Enzyme conjugate(µl): 20, 40, 60, 80, 100 Diluent(ml): 1, 2, 3, 4, 5
3	Add conjugate	100µl
4	Cover the plate and incubate	90 mins at 37°C
5	Wash	6 cycles
6	Prepare chromogenic substrate	No of strips: 1, 2, 3, 4, 5 TMB substrate(ml): 0.8, 1.6, 2.4, 3.2, 4.0 Diluent(ml): 0.8, 1.6, 2.4, 3.2, 4.0
7	Add substrate	150 µl
8	Incubate in dark	30 mins at room temperature
9	Add stop solution	100 µl
10	Read the results	450 nm. /630 nm.

Calculation of results:

Test validity: acceptance criteria [100]:

1. Negative control optical density must be < 0.3
2. Positive control optical density must be > 0.1
3. Mean calibrator optical density must be ≥ 0.35
4. Cut off value must be ≥ 1.5 × Negative control optical density.
5. Ratio of Positive control optical density /cut off must be > 1.1

Calculation[100]:

Sample optical density ratio = Sample optical density / cut off value.

Interpretation of results:

Table no.9Interpretation [100]

S.no	Dengue NS1 antigen units	Interpretation
1	< 9	Negative for Dengue NS1 antigen
2	9 – 11	Equivocal for Dengue NS1 antigen
3	> 11	Positive for Dengue NS1 antigen

Dengue IgM antibody by MICROLISA (J. Mitra):

Principle :

Dengue IgM MICROLISA is an immunoassay based on " MAC Capture ELISA". The microtiter wells were coated with anti human IgM antibodies which bind to the dengue antibodies present in the serum. The dengue antigen conjugate was

added which bind to the specific IgM antibodies. A blue colour was formed when substrate containing chromogen and hydrogen peroxide was added. The Stop solution was finally added to stop the colour reaction and the enzyme substrate reaction was read using EIA reader for absorbance at a wavelength of 450 nm [101].

Table no.10 components of the kit [101]

S.no.	Component	Description
1	Microwells	Coated with anti-human IgM antibodies.
2	Sample Diluent	Buffer containing protein stabilizers and antimicrobial agents.
3	Enzyme conjugate concentrate (10x)	Dengue antigen labelled with horseradish peroxidase with protein stabilizers.
4	Conjugate diluent	Buffer containing stabilizers
5	Wash buffer concentrate(25x)	phosphate buffer solution with surfactant. Dilute 1:25 with distilled water before use
6	TMB substrate	TMB, to be diluted with TMB diluent before use.
7	TMB diluent	Buffer solution containing hydrogen peroxide with preservatives.
8	Positive control	Positive for dengue IgM antibodies with preservative.
9	Negative control	Normal human serum negative for Dengue antibodies with preservative.
10	Calibrator	Positive for dengue IgM antibodies with preservative.

11	Stop solution	Ready to use, 1N Sulphuric acid.

Procedure:

Table no.11 [101]

S.no.	Procedure	Reagents
1	Dilute serum sample	10 µl of sample, 1ml of sample diluent.
2	Add diluted samples and controls/calibrator	100 µl
3	Cover the plate and incubate	60 mins at 37°C
4	Wash	5 cycles
5	Prepare working conjugate	No of strips: 1, 2, 3, 4, 5 Enzyme conjugate(ml): 0.1, 0.2, 0.3, 0.4, 0.5 Diluent(ml): 0.9, 1.8, 2.7, 3.6, 4.5
6	Add conjugate	100µl
7	Cover the plate and incubate	60 mins at 37°C
8	Wash	5 cycles
9	Prepare working substrate	No of strips: 1, 2, 3, 4, 5 TMB: 0.5, 1.0, 1.5, 2.0, 2.5

		substrate(ml)					
		Diluent(ml)	0.5	1.0	1.5	2.0	2.5
10	Add substrate	100 µl					
11	Incubate in dark	30 mins at room temperature					
12	Add stop solution	50 µl					
13	Read the results	450 nm. /630 nm.					

Calculation of results:

Test validity: acceptance criteria [101]:

1. Negative control optical density must be < 0.3
2. Positive control optical density must be > 0.1
3. Mean calibrator optical density must be ≥ 0.35
4. Cut off value must be ≥ 1.5 × Negative control optical density.
5. Ratio of Positive control optical density /cut off must be > 1.1

Calculation [3]:

Sample optical density ratio = Sample optical density / cut off value.

Interpretation of results:

Table no. 12 Interpretation[101]

S.no	**Dengue IgM antibody units**	**Interpretation**
1	< 9	Negative for Dengue IgM antibody
2	9 – 11	Equivocal for Dengue IgM antibody

3	> 11	Positive for Dengue IgM antibody

Lepto IgM antibody- MICROLISA (J. Mitra):

Principle:

Lepto IgM Microlisa is an enzyme immunoassay based on " Indirect ELISA". Microtiter wells are coated with recombinant proteins mixture of various molecular weight. IgM antibodies specific to leptospira binds to the specific leptospira antigens present in the wells. Horseradish peroxidase conjugated Anti-Human IgM was added to the well which binds to the antigen-antibody complex. A blue colour was formed when substrate containing chromogen and hydrogen peroxide was added. The Stop solution was finally added to stop the colour reaction and the enzyme substrate reaction was read using EIA reader for absorbance at a wavelength of 450 nm[102].

Components of the kit:

Table no.13 components of the kit [102]

S.no.	Component	Description
1	Microwells	Coated with leptospira antigens.
2	Sample diluent	Buffer containing protein stabilizers and antimicrobial agents as preservatives.
3	Enzyme conjugate concentrate (50x)	Anti-human IgM labelled with horseradish peroxidase with protein stabilizers.

4	Conjugate diluent	Buffer containing stabilizers.
5	Wash buffer concentrate(25x)	PBS with surfactant. Dilute 1:25 with distilled water before use.
6	Rf absorbent	Buffer containing anti-human IgG antibodies with preservatives.
7	TMB substrate	TMB solution
8	TMB diluent	Buffer solution containing hydrogen peroxide with preservatives.
9	Positive control	Ready to use, Non-infectious synthetic control, positive for Leptospira IgM antibodies and contain sodium azide as preservative.
10	Negative control	Ready to use, Normal human serum negative for Lepto antibodies.
11	Stop solution	Ready to use, 1N Sulphuric acid.

Procedure:

Table no.14 [102]

S.no.	Procedure	Reagents
1	Samples treatment	10 µl of sample, 100 µl of Rf Absorbent.
2	Add controls	125 µl
3	Add sample diluent	100 µl

<table>
<tr><td>4</td><td>Add treated sample</td><td>25 µl</td></tr>
<tr><td>5</td><td>Cover the plate and incubate</td><td>30 mins at 37°C</td></tr>
<tr><td>6</td><td>Wash</td><td>5 cycles</td></tr>
<tr><td>7</td><td>Prepare working conjugate</td><td><table>
<tr><td>No of strips</td><td>1</td><td>2</td><td>3</td><td>4</td><td>5</td></tr>
<tr><td>Enzyme conjugate(ml)</td><td>20</td><td>40</td><td>60</td><td>80</td><td>100</td></tr>
<tr><td>Diluent(ml)</td><td>1</td><td>2</td><td>3</td><td>4</td><td>5</td></tr>
</table></td></tr>
<tr><td>8</td><td>Add conjugate</td><td>100µl</td></tr>
<tr><td>9</td><td>Cover the plate and incubate</td><td>30 mins at 37°C</td></tr>
<tr><td>10</td><td>Wash</td><td>5 cycles</td></tr>
<tr><td>11</td><td>Prepare working substrate</td><td><table>
<tr><td>No of strips</td><td>1</td><td>2</td><td>3</td><td>4</td><td>5</td></tr>
<tr><td>TMB substrate(ml)</td><td>0.5</td><td>1.0</td><td>1.5</td><td>2.0</td><td>2.5</td></tr>
<tr><td>Diluent(ml)</td><td>0.5</td><td>1.0</td><td>1.5</td><td>2.0</td><td>2.5</td></tr>
</table></td></tr>
<tr><td>12</td><td>Add substrate</td><td>100 µl</td></tr>
<tr><td>13</td><td>Incubate in dark</td><td>30 mins at room temperature</td></tr>
<tr><td>14</td><td>Add stop solution</td><td>50 µl</td></tr>
<tr><td>15</td><td>Read the results</td><td>450 nm. /630 nm.</td></tr>
</table>

Calculation of results:

Test validity: acceptance criteria [102]:

1. Blank must be < 0.100 in case of differential filter being used.
2. Negative control optical density must be < 0.3.
3. Positive control optical density must be > 1.1.

Calculation[102]:

Sample optical density ratio = Sample optical density / cut off value.

Interpretation of results:

Table no.15 Interpretation [102]		
S.no	**Lepto IgM units**	**Interpretation**
1	< 9	Negative for Lepto IgM antibodies
2	9 – 11	Equivocal for Lepto IgM antibodies
3	> 11	Positive for Lepto IgM antibodies

Immunochromatographic Test (Lateral Flow Assay)

STANDARD Q Chikungunya IgG/IgM test (SD)

Principle:

The test kit consists of “M”, “G” test lines which are coated with Monoclonal anti-human IgM and Ig G along with “C” control line. Inactivated chikungunya virus in the antigen pad and monoclonal anti-chikungunya E1-gold in the conjugate pad release by adding assay diluent and reacts with anti chikungunya IgM or IgG in patient serum. The serum was added and if human anti-Chikungunya IgM or

IgG was present, a complex is formed consisting of anti-human IgM /IgG, human IgM /IgG, inactivated chikungunya virus and anti-chikungunya E1-gold, a violet band develops[103].

Procedure:

1. A volume of 10 µl of serum is drawn using a micropipette or specimen transfer device and added to the well of the cassette.
2. About 3 drops (90 µl) of buffer is added to the buffer well of the cassette.
3. The result is read after 15 minutes and within 30 minutes [103].

Interpretation:

Table no.16 Interpretation[103]

S.no.	Reading	Result
1	Band only in control line	Negative
2	Two coloured bands (control line and "M" test line)	ChikungunyaIgM positive
3	Two coloured bands (control line and "G" test line)	Chikungunya IgG positive
4	Three coloured bands(control line,"G" test line and "M" test line)	Chikungunya IgG and IgM positive
5	Control band – not visible	Invalid test

Q Malaria P.f / Pan Ag Test (SD)

Principle: The test shows three letters – "P.f" , "pan" as test lines and "C" as control line on the nitrocellulose membrane, which are coated with highly selective individual

antibodies to Histidine-rich protein – II (HRP-II) from Plasmodium falciparum and Lactate dehydrogenase (pLDH) from Plasmodium species. If Histidine-rich protein – II (HRP-II) and Lactate dehydrogenase (pLDH) are present in the serum, a coloured band appears in the result window[104].

Procedure:

1. A volume of 5 µl of serum is drawn using an inverted cup, by dipping the circular end of the inverted cup into the sample and the serum sample is added to the well of the test device.
2. About 3 drops (90 µl) of assay diluent is added to the assay diluent well of the test device.
3. The result is read after 15 minutes and within 30 minutes[104].

Interpretation:

Table no.17 Interpretation [104]

S.no.	Reading	Result
1	Band only in control line	Negative
2	Two coloured bands (control line and "P.f" test line)	Plasmodium falciparum positive
3	Two coloured bands (control line and "Pan" test line)	Plasmodium species (Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae) positive
4	Three coloured bands(control	Plasmodium falciparum positive and

	line, “P.f” test line and “Pan” test line)	Plasmodium species (Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae) positive
5	Control band – not visible	Invalid test

Standard Q Tsutsugamushi IgM/IgG test (SD)

Principle:

The test system consists of a nitrocellulose membrane (NCM) and an absorbent pad. The NCM was coated with antihuman immunoglobulins at the control line and with monoclonal anti-human IgG and monoclonal anti-human IgM at control line 1 and 2. The serum was added and if Tsutsugamushi IgM or IgG was present, the solution reacts with recombinant tsutsugamushi antigen conjugated to gold colloid and a violet band develops [105].

Procedure:

1. A volume of 10 µl of serum is drawn using a micropipette or specimen transfer device and added to the well of the cassette.
2. About 3 drops (90 µl) of buffer is added to the buffer well of the cassette.
3. The result is read after 15 minutes and within 30 minutes[105].

Interpretation:

Table no.18 Interpretation [105]

S.no.	Reading	Result
1	Band only in control line	Negative
2	Two coloured bands (control line and "M" test line)	IgM positive
3	Two coloured bands (control line and "G" test line)	IgG positive
4	Three coloured bands(control line,"G" test line and "M" test line)	IgG and IgM positive
5	Control band – not visible	Invalid test

Heterophile slide agglutination test for scrub typhus (TULIP DIAGNOSTICS):

Weil- Felix test:

Principle:

It works on the principle of antigenic cross reactivity. Group specific alkali stable polysaccharide antigens found in some rickettsiae is shared by some strains of *Proteus* (OX19, OX2 and OXK strains). The rickettsial antigens are detected by using *Proteus* antigens as a suspension of Proteus which readily agglutinates when mixed with serum containing antirickettsial antibodies. The antigen suspension is mixed with the patient's serum. Upon presence, the antibodies against the rickettsial infection reacts with the antigen to produce agglutination reaction [106].

Reagent used:

The reagents contain ready to use, standardized, killed, stained, smooth specific antigen suspensions of *Proteus* OXK/OX19/OX2[106].

Quality Control:

Known sera that are positive and negative for anti-Proteus antibodies should be used [106].

Test procedure: Rapid Heterophile slide agglutination test:

Table no.19 [106]

S.no.	Procedure
1	Place a drop of positive control on the reaction circle of the glass slide.
2	Place a drop of physiological saline on the next reaction circle
3	Place a drop of patient's serum on the next reaction circle
4	Place a drop of appropriate antigen suspension to the reaction circle containing positive control and physiological saline.
5	Place a drop of appropriate antigen suspension to the reaction circle containing patient's serum.
6	Mix the contents of the circle uniformly with separate match sticks.
7	Rock the slide back and forth and observe for agglutination at one minute.

Result [106]:

Positive result: Agglutination obtained within one minute.

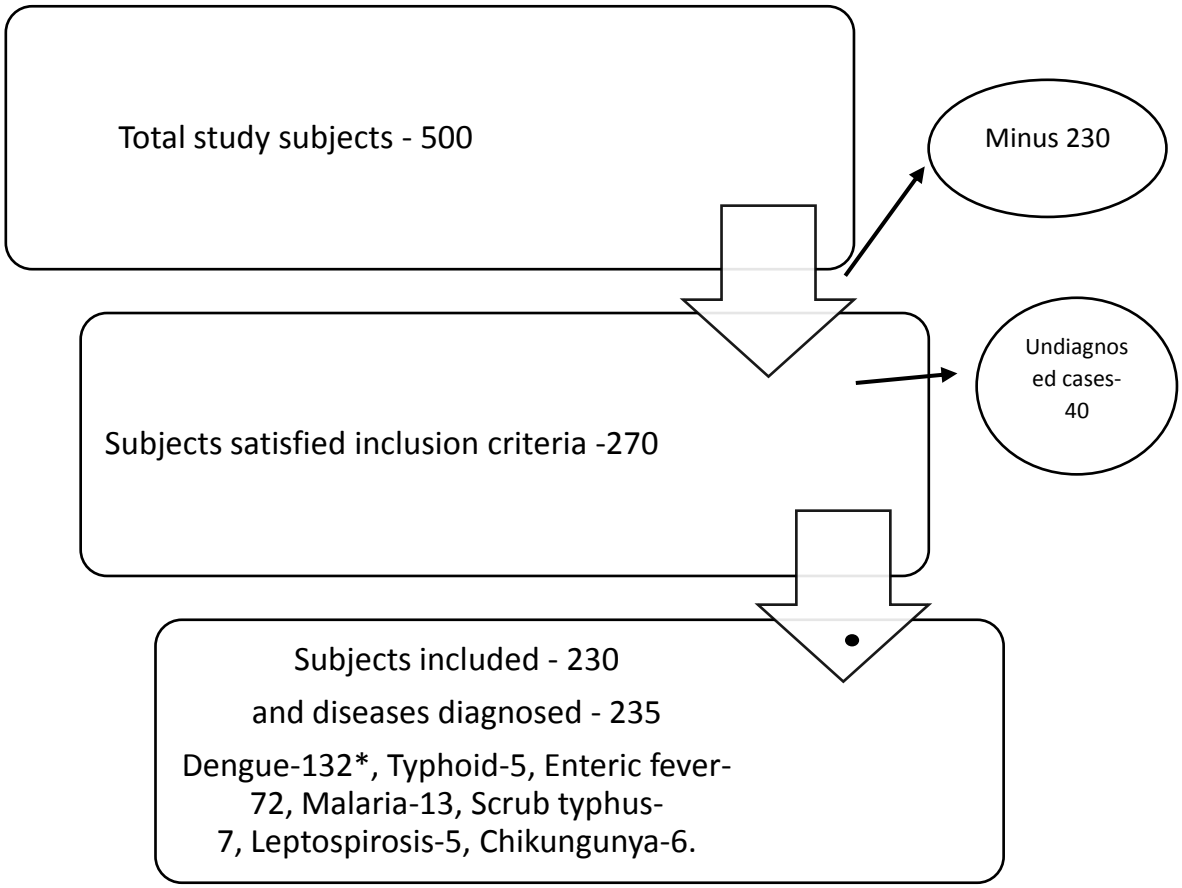
Negative result: no agglutination.

Analysis of results:

- In epidemic and endemic typhus – agglutination is seen in OX19, sometimes in OX2.
- In tickborne spotted fever – agglutination in OX19 and OX2.
- In scrub typhus – agglutination in OXK.
- Negative result - rickettsial pox, Q fever, ehrlichiosis and bartonellosis

5.0. Results:

"A CLINICOMICROBIOLOGICAL STUDY ON INFECTIVE CAUSES OF ACUTE FEBRILE ILLNESS" was carried out in the Central Laboratory of Department of Microbiology at Trichy SRM Medical College and Research Centre, Trichy. out of 500 patients admitted in various medical wards for acute febrile illness during the study period of consecutive 12 months (January to December 2018). Among them, the causes for AUFI was diagnosed in 230 cases. Five cases of Dengue fever also had Enteric fever. Hence total cases included were 230 and diseases diagnosed were 235. The flow diagram of the total cases is given in figure no.11. The results of these 230 cases are furnished in the ensuing pages.



* Concurrent / Co-infection – Dengue and enteric fever in 5 cases

Figure 11: Flowchart of the study subjects

When a battery of selected tests were carried out to diagnose AUFI based on the syndromic approach for the 270 cases, diagnosis could not be made out in 40 of the 270 cases. The epidemiological parameters, clinical and microbiological aspects of the 230 cases are furnished in the tables and figures given below.

5.1. Genderwise distribution:

Among the 230 cases, 119 (51.74%) were males and 111 (48.3%) were females.

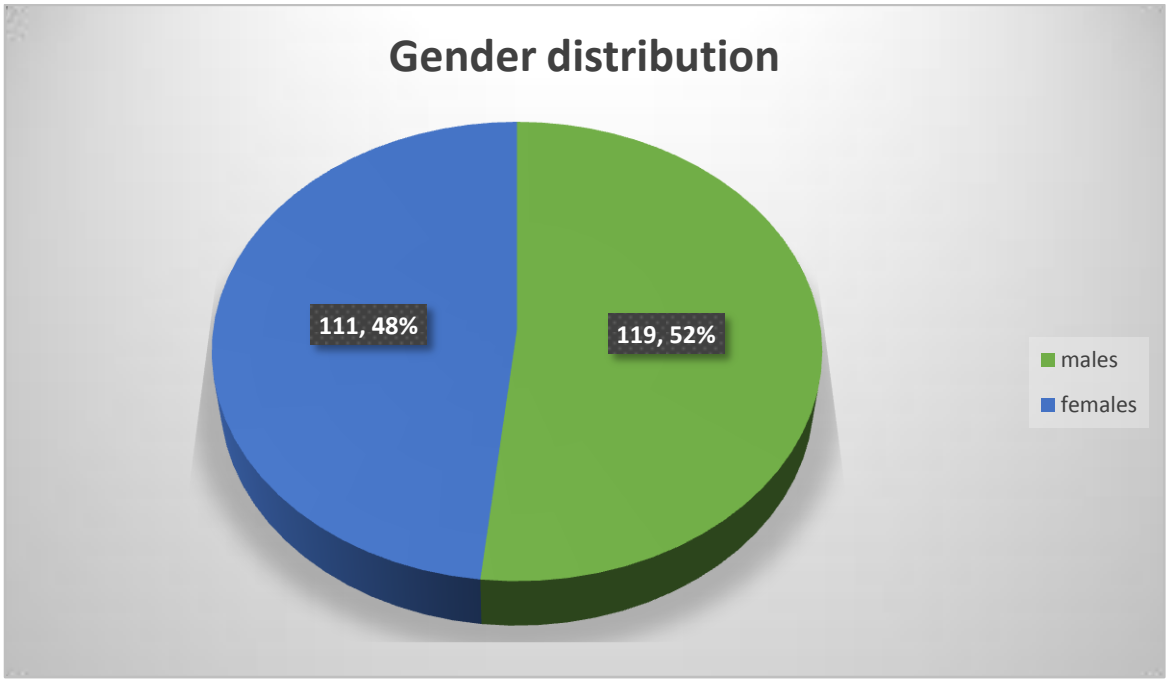


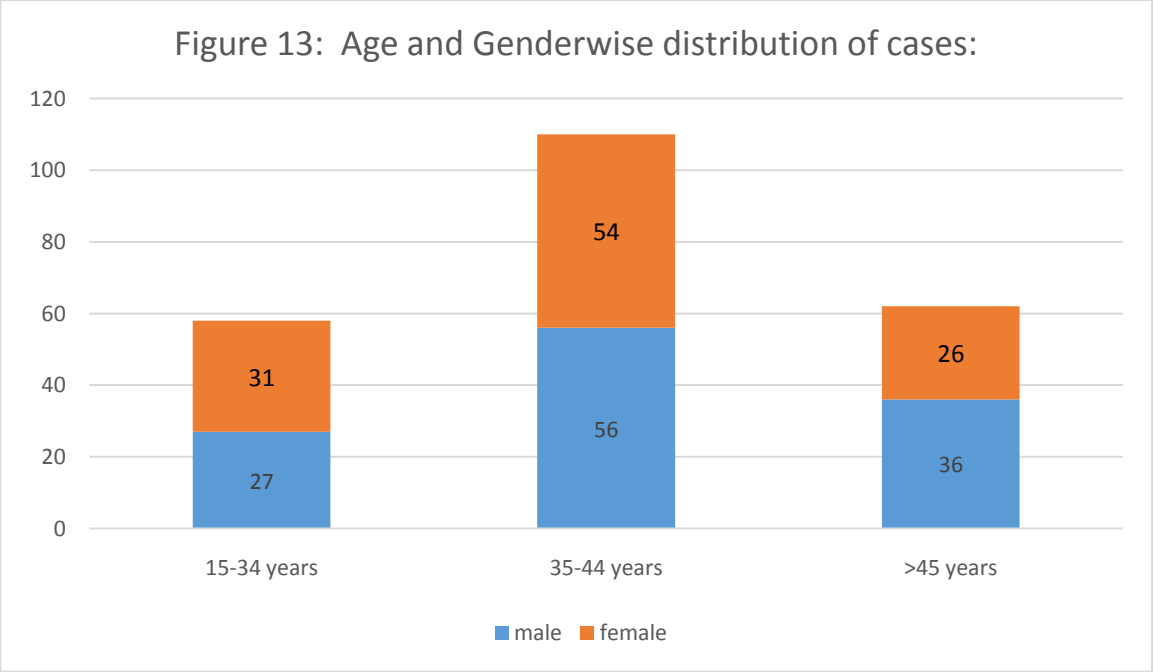
Figure 12: Genderwise distribution

5.2. Age and genderwise distribution:

The cases included in the study were above the age group of 15 years. The age range of the study subjects varied from 15 to 88. The mean affected age was 40.9 years. The mean age of the males and females were 39.8 and 42.5 years respectively. Overall, significant (p=<0.01) number of cases were seen above the age group of 35 and above. There was no significant difference in age and gender when the distribution of cases was classified

and analysed in relation to 3 groups along with gender. The distribution of cases in relation to age group is given in Table 20 and Figure 2.

Table 20: Agewise distribution				
S.no	**Age group**	**Male(%)**	**Female(%)**	**Total(%)**
1	15 - 34 years	27(11.4)	31(13.2)	58(25.22)
2	35 - 44 years	56(24.35)	54(23.4)	110(47.83)
3	≥ 45 years	36(15.65)	26(11.3)	62(26.96)
	Total	119 (51.74)	111 (48.3)	230

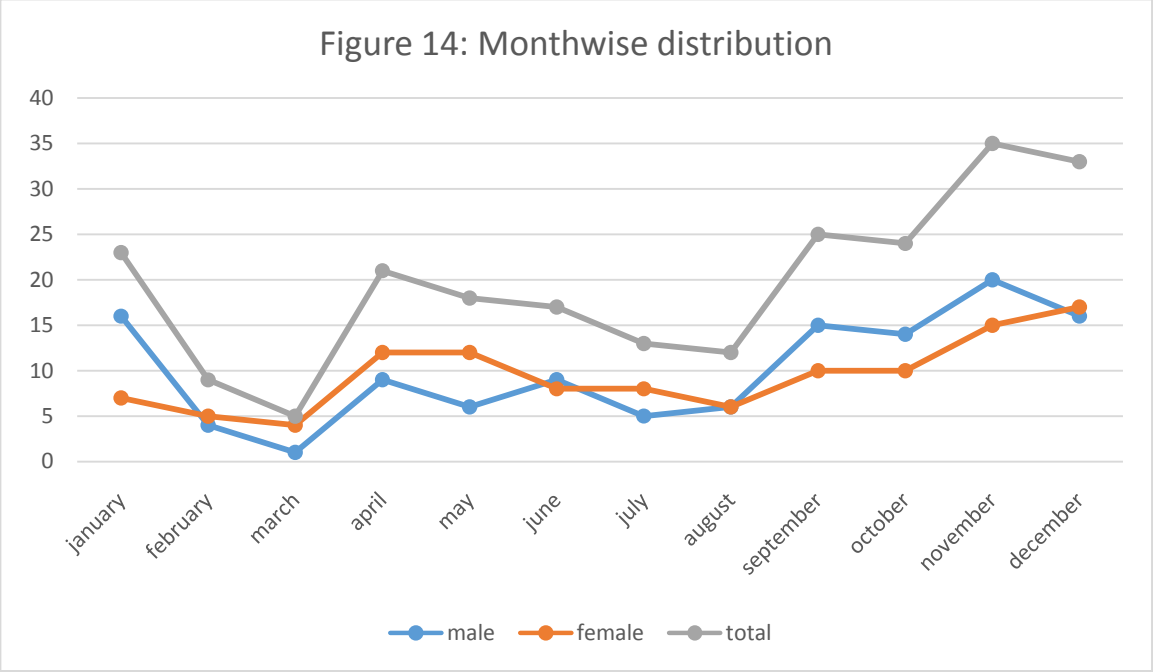


5.3. Monthwise distribution:

A total of 230 cases were analysed in relation to the month and gender and the distribution is given in Table 21 and Figure 14. Cases were seen more from the period of September to December and in the month of January. These variations tallied with vectors responsible for transmission and water contamination in monsoon period.

Table 21: Monthwise distribution				
S.no	**Month of the year (2018)**	**Male**	**Female**	**Number(%)**
1	January	16	7	23 (9.8)
2	February	4	5	9 (3.8)
3	March	1	4	5 (2.1)
4	April	9	10	19(8.3)
5	May	6	11	17(7.2)
6	June	7	8	15(6.52)
7	July	7	8	13(5.5)
8	August	6	6	12(5.1)
9	September	15	10	25(10.6)
10	October	14	10	24 (10.2)
11	November	20	15	35 (14.9)
12	December	16	17	33 (14)
	Total	119	111	230

During study period, we noticed increased occurrence of AUFI during post monsoon period and continues during winter, even though cases of AUFI were seen all over the year.



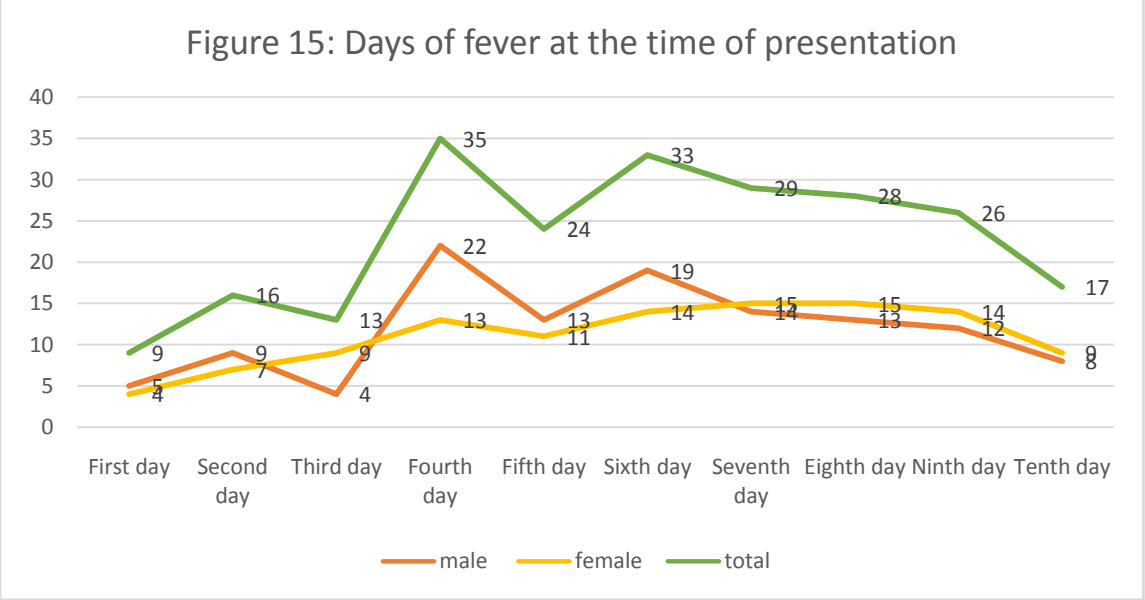
5.4. Time of presentation of fever:

Distribution of cases in relation to duration of fever and gender are depicted in Table 22 and Figure 15. When an analysis was made between male and female in relation to the duration of fever at presentation to the hospital, no significance was noted. The observations indicate that females come to hospital later than males after the onset of fever. The table below shows the distribution of presentation in relation to duration.

Table 22: Time of presentation of fever in relation to duration.

S.no	Presentation of fever	male	female	Number(%)
1	First day	5	4	9 (3.8)
2	Second day	9	7	16(6.96)
3	Third day	4	9	13 (5.5)
4	Fourth day	22	13	35 (14.9)
5	Fifth day	13	11	24 (10.2)
6	Sixth day	19	14	33 (14)

7	Seventh day	14	15	29 (12.3)
8	Eighth day	13	15	28 (11.9)
9	Ninth day	12	14	26 (11.06)
10	Tenth day	8	9	17 (7.2)
Total		119	111	230



5.5. Symptoms and Signs:

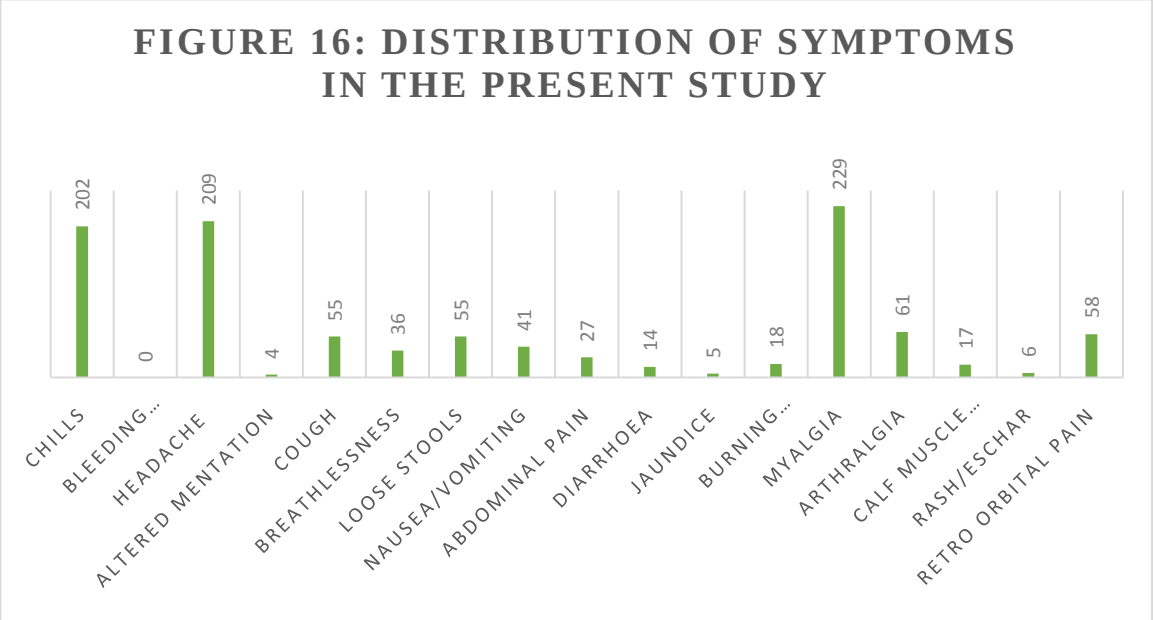
The symptoms and signs noticed among the 230 cases are depicted in Table 23 and in Figure 16 and 17. If any symptom was noticed in more than 75% of cases other than fever, it was considered as the commonest symptom for study purpose.

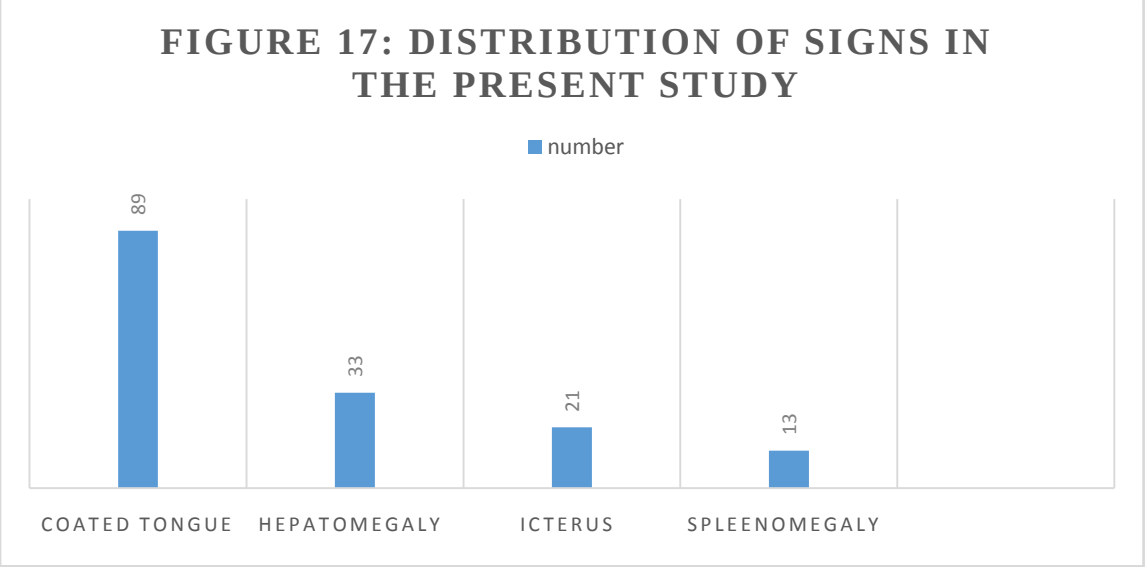
Table 23: Symptoms and Signs

Systemwise	Symptoms	Number (%)*
Generalised	Chills	202 (85.9)
	Bleeding manifestation	0
Central nervous system	Headache	209 (88.9)
	Altered mentation	4 (1.7)
Respiratory system	Cough	55 (23.4)

	Breathlessness	36 (15.3)
Gastrointestinal system	Loose stools	55 (23.4)
	Nausea/vomiting	41 (17.4)
	Abdominal pain	27 (11.5)
	Diarrhoea	14 (5.9)
	Jaundice	5 (2.13)
Genitourinary system	Burning micturition	18 (7.7)
Musculoskeletal system	Myalgia	229 (97.4)
	Arthralgia	61 (25.9)
	Calf tenderness	17 (7.23)
Skin	Rash/eschar	6 (2.56)
Ophthalmic system	Retro orbital pain	58 (24.7)
	Signs	**Number (%)***
Gastrointestinal system	Coated tongue	89 (37.9)
	Hepatomegaly	33 (14.04)
	Splenomagaly	13 (5.53)
Others	Icterus	21 (8.93)
* The number overlapping with each other		

Under this category, frequently seen symptoms were myalgia, headache and chills among 97.4, 88.9 and 85.9 % respectively. The other symptoms elicited among the cases are given in Table 23. The commonest symptom noticed among these cases were independent of gender and in the order of myalgia, headache and chills. The signs elicited among the 230 cases were coated tongue, hepatomegaly, splenomegaly and icteus in 89(37.97%), 33(14.04%), 21(8.93%) and 13(5.53%) respectively.

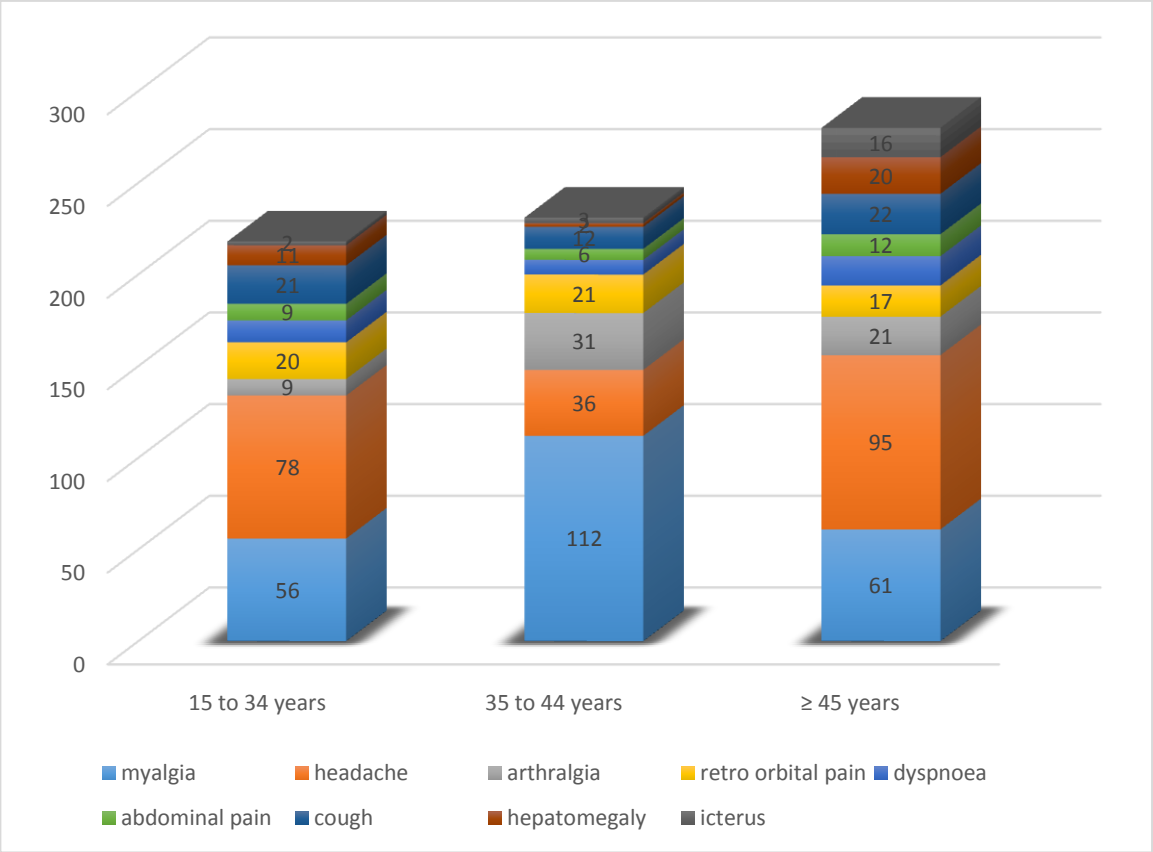




5.6. Distribution of symptoms in relation to age group and gender:

The commonest symptom noticed among these cases irrespective of gender were in the order of myalgia, headache and chills and these were independent of gender. When analysis of these symptoms in relation to age group was done, myalgia was seen more among the age group 35-44 years whereas headache was noticed more among the age group 15-34, and 45 and above, and chills was noticed equally among all the three age groups. The details are shown in Figure 18.

Figure 18: Distribution of symptoms in relation to age group:



5.7. Distribution of cases in relation to diseases:

The diseases diagnosed by laboratory means among the 230 cases are depicted in Table 24 and Figure 19 .

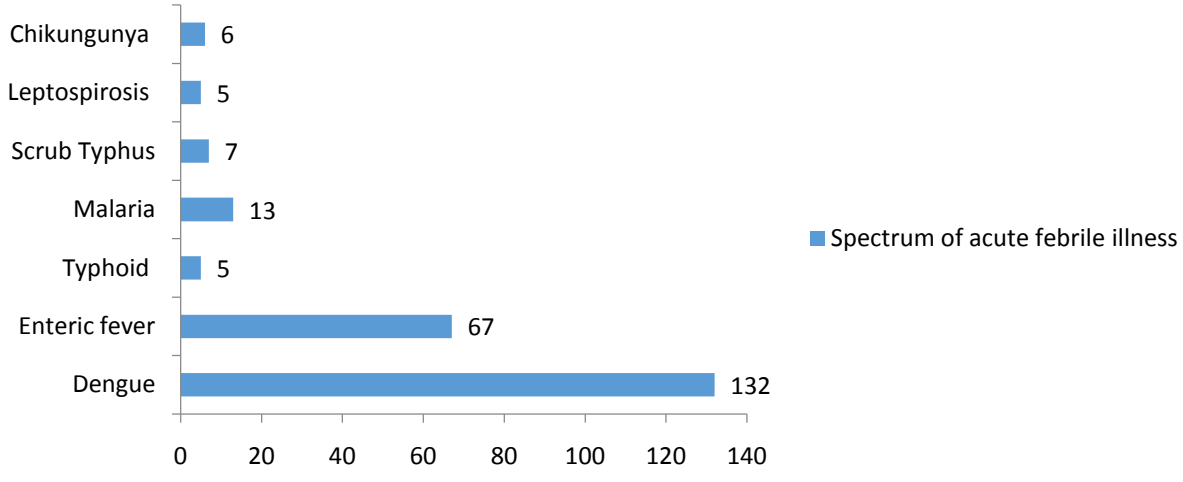
Table 24: Distribution in relation to diseases diagnosed(n=230)				
S.no	Category		Diseases (AUFI)	Total(%)
1	Water borne diseases		Enteric fever	67 (29.13)
			Typhoid	5(2.173)
2	Vector borne diseases	Aedes	Dengue*	132 (56.2)

		aegypti	Chikungunya	6 (2.55)
		Anopheles species	Malaria	13 (5.53)
		Trombiculid mite	Scrub typhus	7 (2.97)
3	Zoonotic diseases		leptospirosis	5 (2.13)

*5 had Enteric fever also.

In the consecutive pages, the results of cases in relation to the 6 causes of acute febrile illness are described. The distribution of disease among the 235 cases were in the order of Dengue, Enteric fever (including S.typhi), Malaria, Scrub typhus, Chikungunya and Leptospirosis in 132 (56.2%), 67 (29.13%), 5(2.17%), 13 (5.53%), 7 (2.97%), 6 (2.55%) and 5 (2.13%) respectively. 5 among them had Dengue and Enteric fever. The details are given in Table 24 and Figure 19. Further descriptions with regard to diseases are given in the ensuing pages.

Figure 19: Spectrum of acute febrile illness



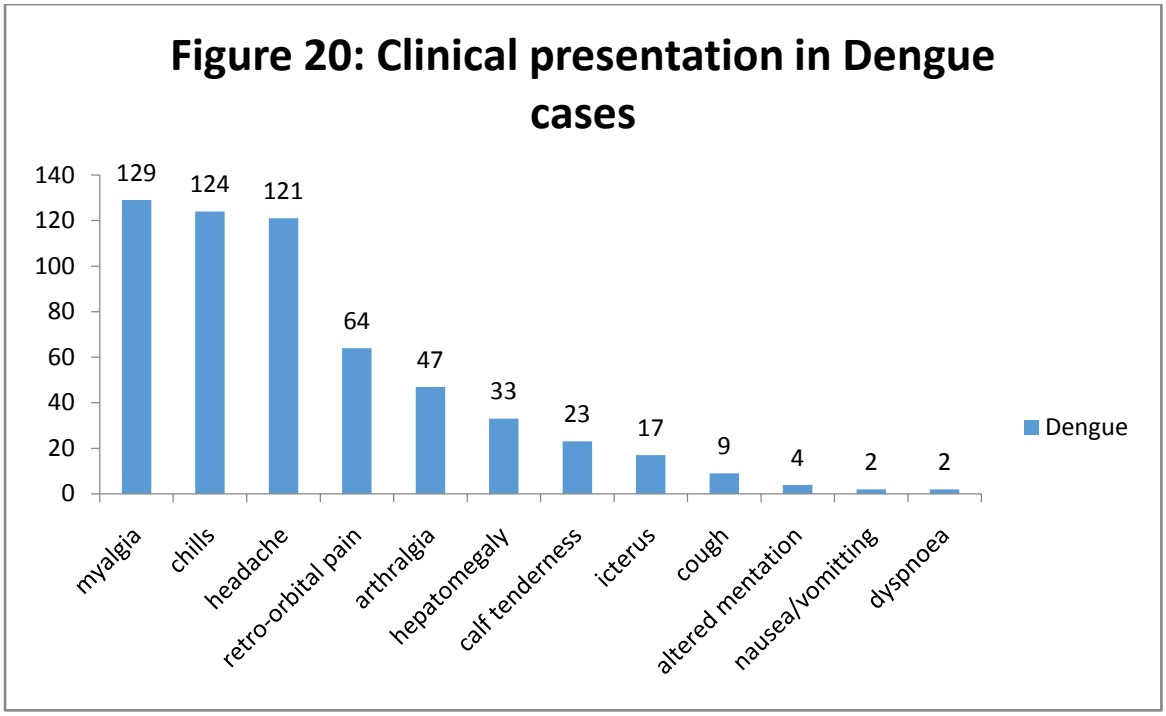
5.8. Clinical aspects of Dengue:

During the study period, Dengue fever was seen in 132 of 230 cases which was the highest one. Distribution of Dengue cases in relation to age and gender are given in Table 25. Male predominance was seen and the male to female ratio was 2.1:1 in the age group 45 and above. The mean affected age was 38 years. Dengue was seen more among the age group 35 and above, but there was no significance in gender when analysed. Five of the dengue cases had unequivocal serological and clinical evidences of Enteric fever. Maximum number of cases were enrolled in the month of November, which tallies with post monsoon period. The most common presenting symptom was myalgia, followed by chills and headache. Figure 20 depicts the clinical presentation seen in dengue cases.

Table 25: Age and Genderwise distribution in dengue cases

S.no	Age group	Male	Female	Total
1	15 - 34 years	15	17	33
2	35 - 44 years	31	34	65
3	≥ 45 years	22	13	35

The clinical symptoms and signs are shown in Figure 20.



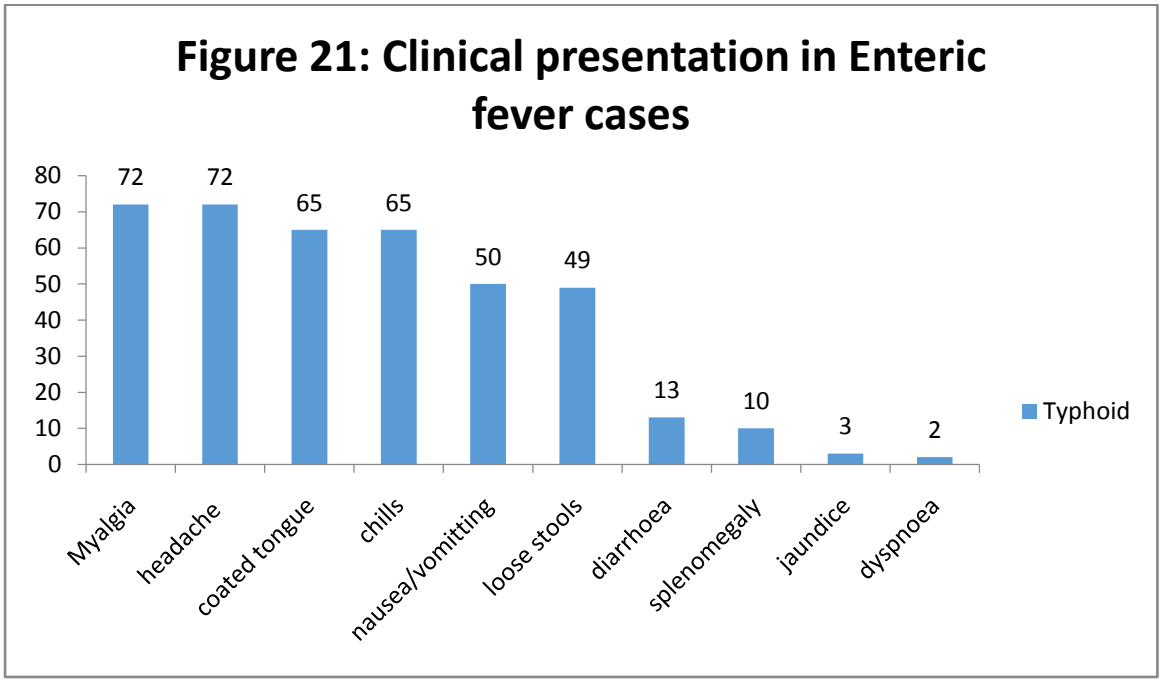
5.9. Clinical aspects of Enteric fever:

During the study period, Typhoid fever was seen in 72 cases which included 5 Blood culture positives and the remaining 67 based on Widal test in which S.typhi 'O' titre was positive with 1:160 dilution or above with one of the clinical symptoms i.e headache along with bedside signs coated tongue, with or without relative bradycardia and other clinical findings splenomegaly, hepatomegaly or hepatospleenomegaly. The blood culture positive cases also showed a S.typhi 'O' titreof 1:160 dilution or above. Maximum number of cases were enrolled in the month of December though cases were documented all over the year. The most common presenting symptom was myalgia, followed by headache. Headache was seen in 51 out of 72 cases but low grade in nature, but intensity was severe in those who had concurrent or co-infection with Dengue. Also, clinical examination revealed splenomegaly in 10 cases, which was soft in nature and hepatomegaly in 23 cases and hepatosplenomegaly in 23 cases. A correlation between clinical findings and microbiological

aspects are given under Microbiology section. Coated tongue was commonly seen in 65 of the 72 cases. Figure 21 depicts the Clinical presentation seen in Enteric fever cases.

Table 26: Age and Genderwise distribution in Enteric fever cases				
S.no	**Age group**	**Male**	**Female**	**Total**
1	15 - 34 years	10	10	20
2	35 - 44 years	20	15	35
3	≥ 45 years	9	8	17

The clinical symptoms and signs are shown in Figure 21.



5.10.Clinical aspects of Malaria:

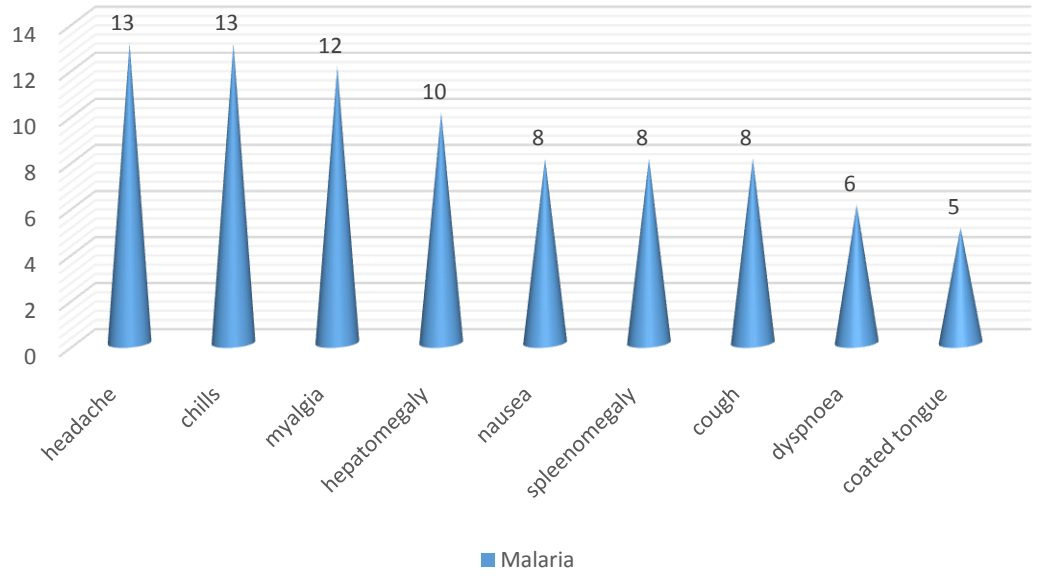
During the study period, Malaria was seen in 13 of 230 cases. Distribution of Malaria cases in relation to age group and gender are given in Table 20. Simple analysis between age group and gender revealed that the disease was distributed almost equally among both genders without any preference to age or gender. However statistical analysis could not be done, since

the numbers available in each cell was small. The malarial cases were recorded occasionally and not on all months of the year. The most common presenting symptom was myalgia, followed by chills and headache. Figure 22 depicts the presentation seen in Malaria cases.

Table 27: Age and Genderwise distribution in Malaria cases				
S.no	**Age group**	**Male**	**Female**	**Total**
1	15 - 34 years	1	2	3
2	35 - 44 years	3	3	6
3	≥ 45 years	2	2	4

The clinical symptoms and signs are shown in Figure 22.

Figure 22: Clinical presentation in Malaria cases



5.11. Clinical aspects of Scrub typhus:

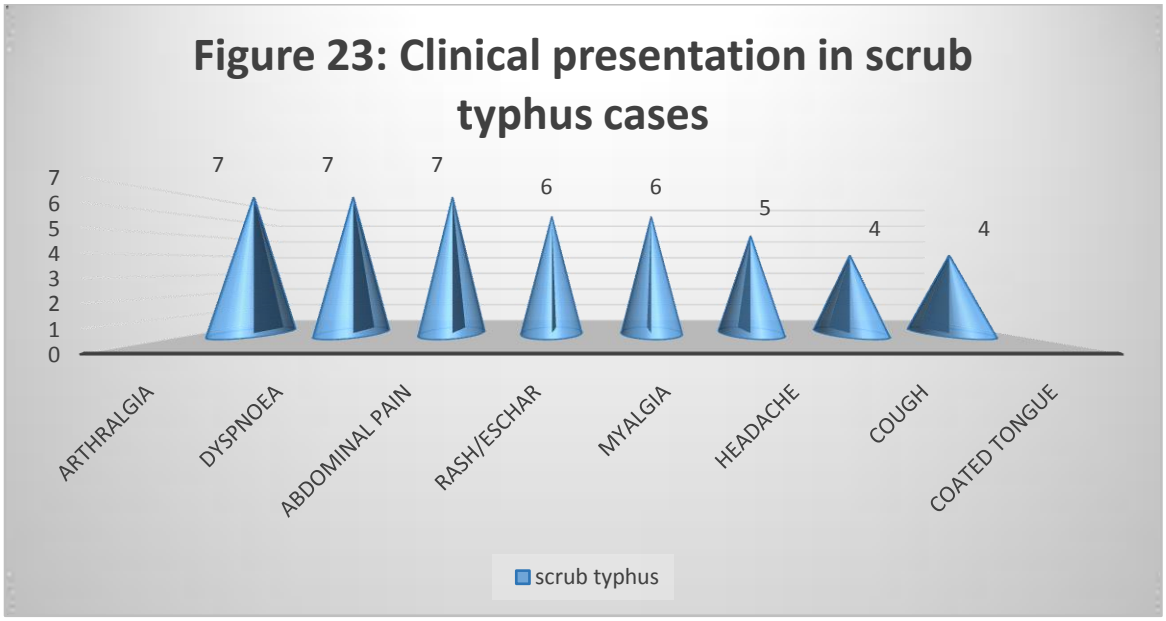
During the study period, Scrub typhus was seen in 7 of 230 cases. Distribution of Scrub typhus cases in relation to age group and gender are given in Table 28. Scrub typhus

was seen in 7 cases in the study period - 3 males and 4 females. Two cases were seen in the age group 35-44 years and 5 in the age group of ≥ 45 years, showing that the occurrence was independent of gender. Eschar was the pathognomic feature of scrub typhus and was seen in 6 of 7 cases. The most common presenting symptom was arthralgia and dyspnoea. Figure 23 depicts the presentation seen in scrub typhus cases. Scrub typhus was not seen all over the year.

Table 28 Age and Genderwise distribution in Scrub typhus cases

S.no	Age group	Male	Female	Total
1	15 - 34 years	0	0	0
2	35 - 44 years	1	1	2
3	≥ 45 years	2	3	5

The clinical symptoms and signs are shown in Figure 23



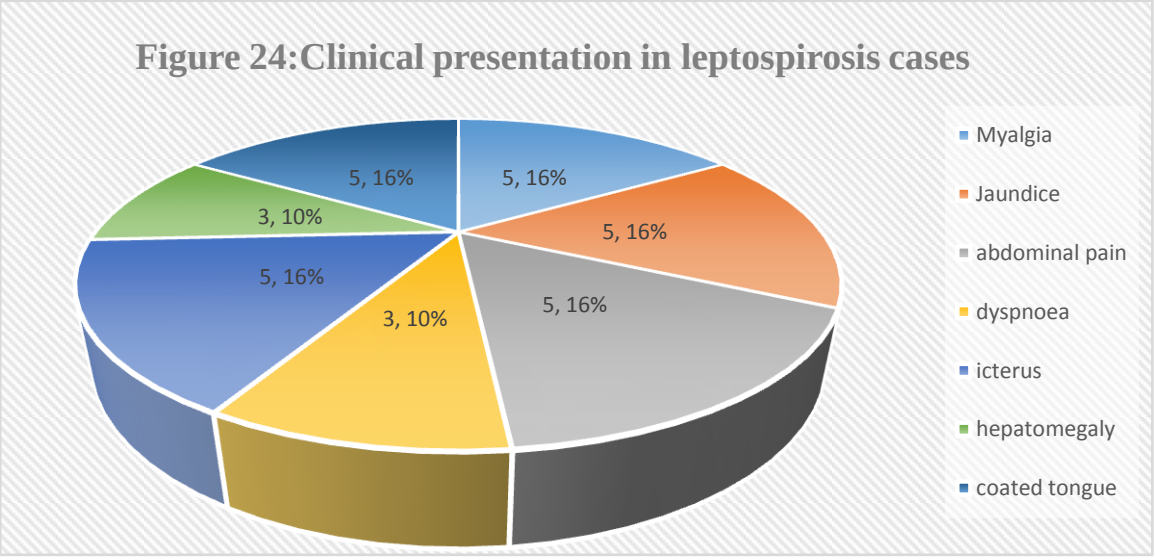
5.12. Clinical aspects of Leptospirosis:

During the study period, Leptospirosis was seen in 5 cases of 230 cases. Distribution of Leptospirosis cases in relation to age group and gender are given in Table 24. It was seen in 2 males and 3 females. One case was seen in the age group 15-34 years, 2 cases in the age group 35-44 years and 2 in the age group of ≥ 45 years, showing that the occurrence was independent of gender. Maximum number of cases were enrolled in the month of January. The most common presenting symptom was myalgia and jaundice. Icterus was the commonest sign among these cases. Figure 29 depicts the clinical presentation seen in Leptospirosis cases.

Table 29: Age and Genderwise distribution in Leptospirosis cases

S.no	Age group	Male	Female	Total
1	15 - 34 years	0	1	1
2	35 - 44 years	1	1	2
3	≥ 45 years	1	1	2

The clinical symptoms and signs are shown in Figure 24



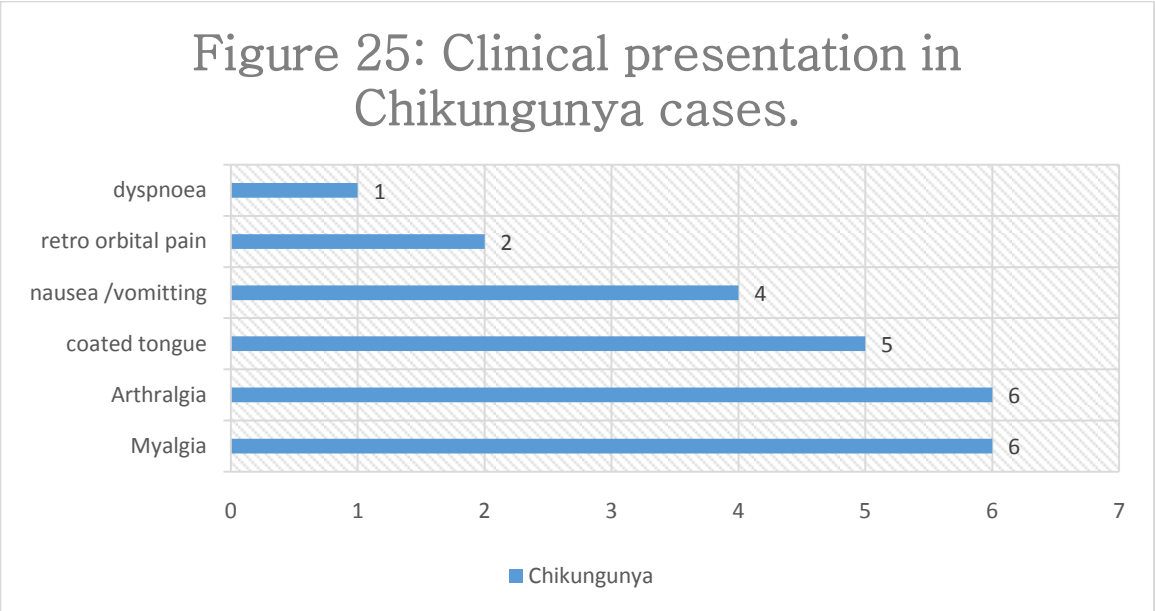
5.13. Clinical aspects of Chikungunya:

During the study period, Chikungunya was seen in 6 out of 230 cases. Distribution of Chikungunya cases in relation to age group and gender are given in Table 30.It was seen in 3 males and 3 females. One case was seen in the age group 15-34 years, 2 cases in the age group 35-44 years and 3 in the age group of ≥ 45 years, showing that the occurrence was independent of gender. Maximum number of cases were enrolled in the month October which tallies with the vectors responsible. The most common presenting symptom was myalgia and arthralgia. Figure 25 depicts the presentation seen in Chikungunya cases.

Table 30: Age and Genderwise distribution in Chikungunya cases

S.no	Age group	Male	Female	Total
1	15 - 34 years	0	1	1
2	35 - 44 years	1	1	2
3	≥ 45 years	2	1	3

The clinical symptoms and signs are shown in Figure 25



5.14. Hematological parameters:

Distribution of the hematological parameters observed among the 230 diagnosed cases are described and shown in Table 31. Hemoglobin was <12 gm/dl in 44(18.7%) cases and 75 cases had Thrombocytopenia (count <1.5 l/cumm). Leucocytosis was seen in 15(6.4), where the Total count was >10000cells/cumm. Haemoglobin was <12 gm/dl in 5 of 13 cases of malaria and remaining in the other cases. Leukopenia was seen in 79% (n=57) of cases and remaining was seen in patients with Dengue. Leucocytosis was seen in 5 cases of Leptospirosis, 7 cases of Dengue fever and one in Scrub typhus. Thrombocytopenia was seen in 4 cases of Malaria and 71 cases of Dengue fever.

Table 31: Hematological parameters

S.no.	Parameters	Number (%) (n=230)
1	Hemoglobin (<12 gm/dl)	44(18.7)
2	Leucocytosis (Total count >10000cells/cumm.)	15(6.4)
3	Leukopenia <3500/cumm	70(30.34)
4	Thrombocytopenia (<1.5 l/cumm.)	75(32.6)

5.15. Microbiological Studies:

5.15.1. Peripheral smear preparation:

Malaria was diagnosed by Peripheral smear in 12 cases. It was negative in other 258 cases, who had AUFI. None of the Malaria patients suffered from dual infections. The details are shown in Table 32.

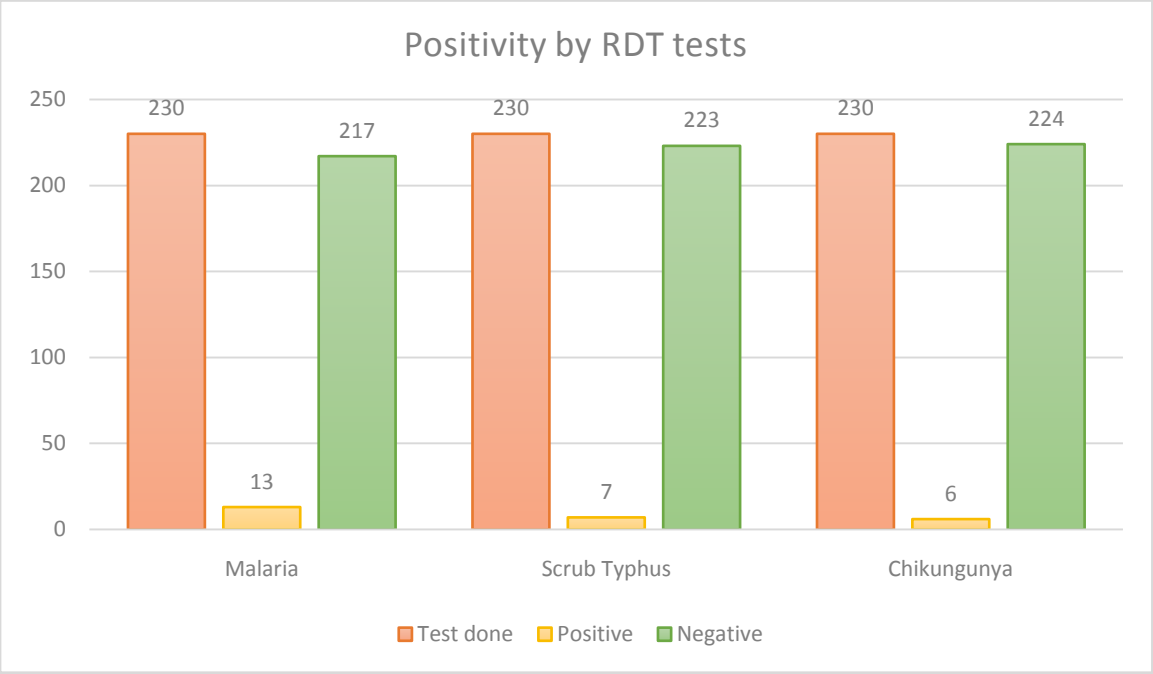
5.15.2 Dark field microscopy:

Routine Dark field microscopy of the plasma of 270 cases helped to recognise Leptospirosis only in 3 cases. The details are shown in Table 32.

5.15.3Rapid diagnostic test (Immunochromatograpy):

Rapid diagnostic test was done for all 235 cases for diagnosing Malaria, Scrub typhus and Chikungunya. 13 cases were positive for Malaria, 7 were positive for Scrub typhus and 6 for Chikungunya and they are shown Figure 26.

Figure 26: Positivity by RDT tests.



5.15.4 ELISA Test:

For diagnosis of Dengue, ELISA Test for NS1 antigen (early) and IgM antibody (after 5 days of fever) was performed. For diagnosis of Leptospirosis, IgM antibody was performed by ELISA. Dengue NS1 antigen and IgM antibody ELISA were positive in 88 and 44 cases respectively and both were positive in 30 cases. Five cases were positive when tested by Lepto IgM antibody ELISA. The positivity by these tests are depicted in Table 32 and Figure 27.

During the study period 97 patients came with history of fever of ≤ 5 days. When NS1 antigen was carried out for 97 patients, it was positive in 88 cases and negative in the remaining 11 cases. Thus the positive rate for the diagnosis of Dengue fever in the study area was 76.5%. Analysis of these 88 cases revealed that there was 39 males and 49 females.

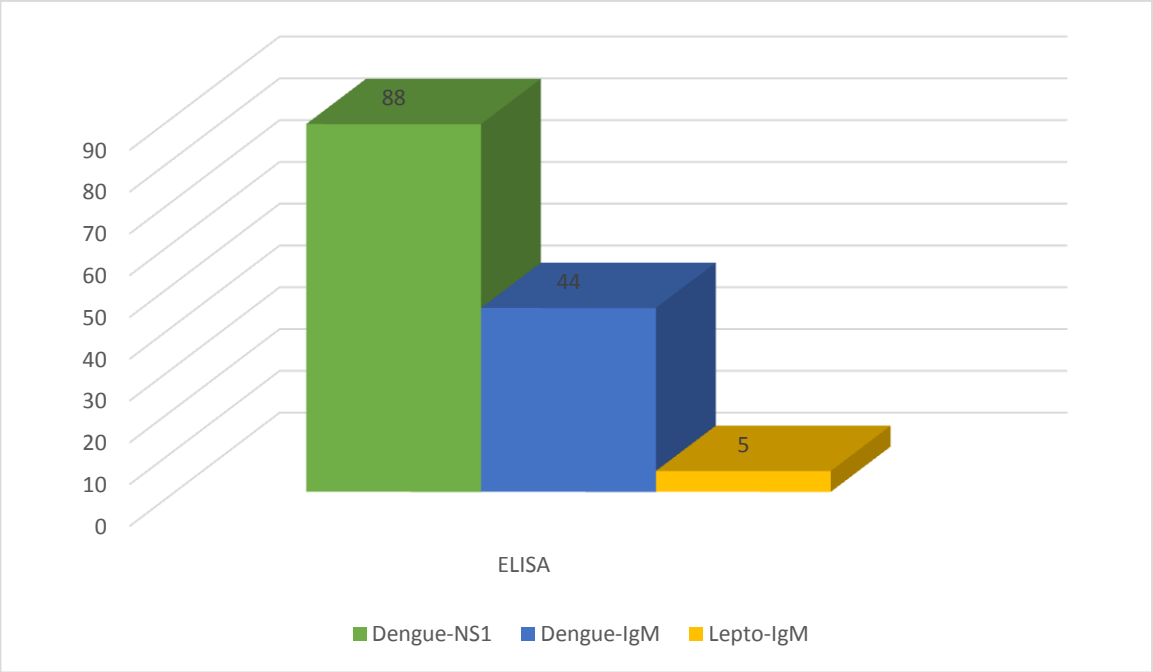
Dengue IgM antibodies was carried out for the remaining 133 of the 230 cases who landed in the hospital with the duration of fever > 5 days. Dengue IgM was positive in 44 of the 133 cases. Thus IgM positive dengue cases during the study period was 28.4%. 30 cases of NS1 antigen positive cases showed IgM ELISA positivity when carried out on the 6^{th} and 7^{th} day of fever.

Table 32: ELISA positive status among 230 cases of AUFI

Name of ELISA	Number tested			Number Positive			Percentage of Positivity		
	M	F	T	M	F	T	M	F	T
Dengue NS1 antigen	44	53	97	39	49	88	16.9	21.3	38.3
Dengue IgM antibody	66	67	133	27	17	44	11.7	7.4	19.1
Lepto IgM antibody	119	111	230	3	2	5	1.30	0.87	2.17

M-male, F-Female, T-Total

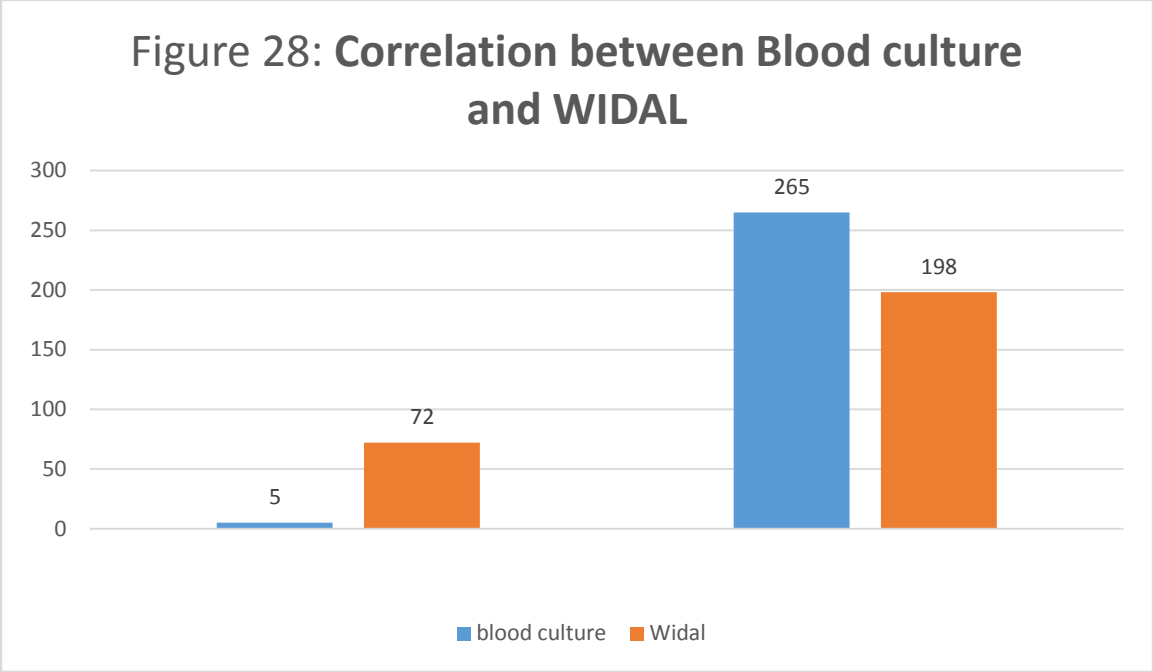
Figure 27: ELISA positivity



5.15.5. Blood culture and WIDAL:

Five Salmonella typhi isolates were obtained from the 270 samples. Among the 5, there was 3 males and 2 females. Though the other blood samples were negative for culture, Widal test was positive in all 72(45.3%) out of the 159 cases who had duration of the fever > 7 days. Among these 72 cases, there were 36 males and 31 females. Repeat Widal test was carried out in the paired serum sample one week after the first sample to look for rise in titre. But it was successfully carried out only in 15 of the 72 diagnosed to have Typhoid fever as the remaining 57 were discharged earlier. Out of 15only three showed four fold rise in titre. The results are furnished in Table 33 given below

Table 33: Correlation between blood culture and WIDAL Total processed: 230		
	Positive	Negative
Blood culture	5(1.85%)	265
WIDAL	72(26.67)	198



5.15.6. Antimicrobial sensitivity test:

The antimicrobial sensitivity test was performed for the 5 isolates of S.typhi. Ampicillin and Amoxycillin were sensitive only in 2 isolates (40%). All the 5 isolates were sensitive to Chloramphenicol, Ciprofloxacin. Ceftriaxone Cefotaxime and Cefipime. The details are given in Table 34.

Table 34: Antibiogram pattern.

Antibiotics	***S.typhi n=5***
Ampicillin	40%
Amoxicillin	40%
Ciprofloxacin	100%
Chloramphenicol	100%
Ceftriaxone	100%
Cefotaxime	100%
Cefipime	100%

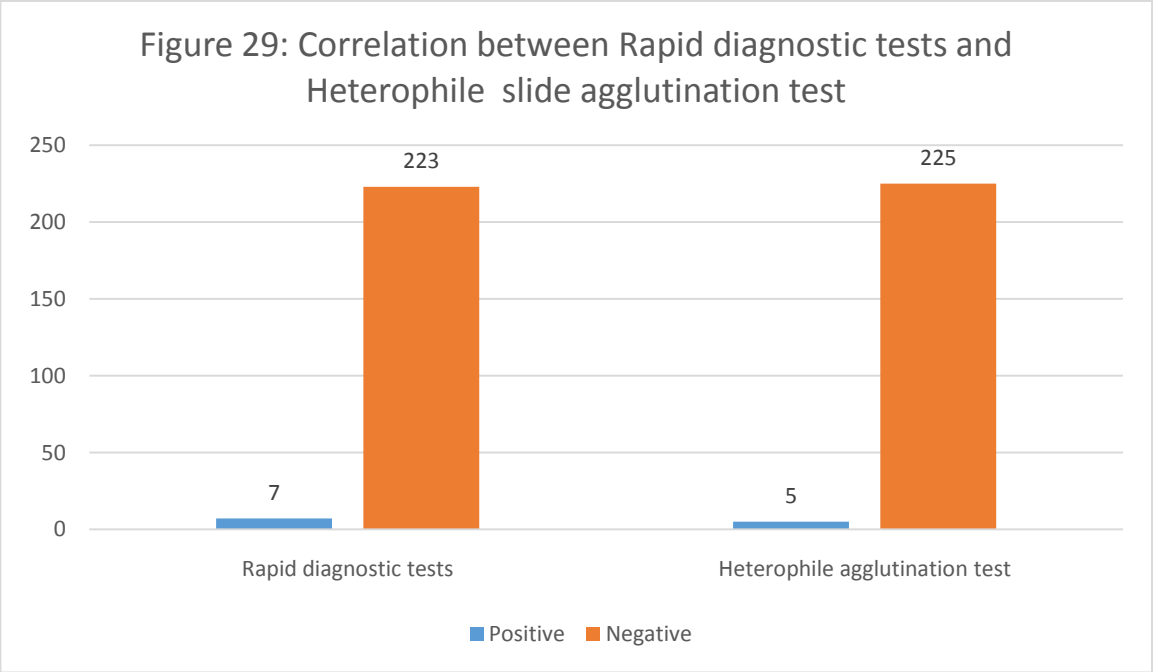
5.15.7. Rapid diagnostic tests and Heterophile slide agglutination test(Weil-felix test):

Rapid diagnostic test (Immunochromatographic test) and Heterophile agglutination test were performed for diagnosing Scrub typhus. Out of 235 cases, 7 cases were positive by Rapid diagnostic test (2.97%) and 5 were positive by Heterophile slide agglutination test (2.13%) and they are shown in Table 35 and Figure 29.

Table 35: Result of Rapid diagnostic tests (Immunochromatographic test) and Heterophile slide agglutination test

	Positive	Negative	Total
Rapid diagnostic tests (Immunochromatograpic test)	7	263	270

Heterophile slide agglutination test	5	265	270



5.16 Co-Infection / Con-current infection:

Co-infection was observed in 5 cases of Dengue and Typhoid (1.9%). Figure 30 depicts the occurrence.

Figure 30: Occurrence of Co-Infection

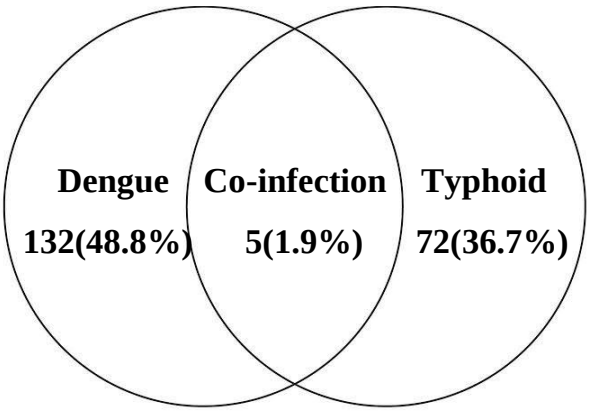


Table 36: Results by Laboratory tests in relation to disease.							
Tests	Dengue	Enteric fever	malaria	Scrub typhus	chikunguny a	leptospirosi s	Total
P.s	-	-	12(4.4)	-	-	-	12(4.4)
DFM	-	-	-	-	-	2(0.74)	2(0.74)
Bld culture	-	5(1.85)	-	-	-	-	5(1.85)
WIDA L 0-1:160 H-1:320	-	72(26.6)	-	-	-	-	72(26.6)
Dengue -NSI ELISA	88(76.5)	-	-	-	-	-	88(76.5)
Dengue IgM ELISA	44(28.4)	-	-	-	-	-	44(28.4)
Lepto IgM ELISA	-	-	-	-	-	5(1.85)	5(1.85)
RDT-Chik	-	-	-	-	6(2.22)	-	6(2.22)
RDT – ST	-	-	-	7(2.59)	-	-	7(2.59)
RDT-Malaria	-	-	13(4.81)	-	-	-	13(4.81)
Weil-felix	-	-	-	5(1.85)	-	-	5(1.85)

5.17. Treatment, Hospital stay and Outcome of the study:

The febrile cases were hospitalised for a duration of 5-10 days depending on the severity of the illness. The mean hospital stay was found to be 6.73 days. The median hospital stay was 5 days. All of the 230 diagnosed cases were provided with appropriate medications and supportive care and all the 230 cases recovered. The clinical course was uneventful and none of the patients developed any untoward events and complications. The Figure 31 given below shows the outcome observed in the febrile cases.

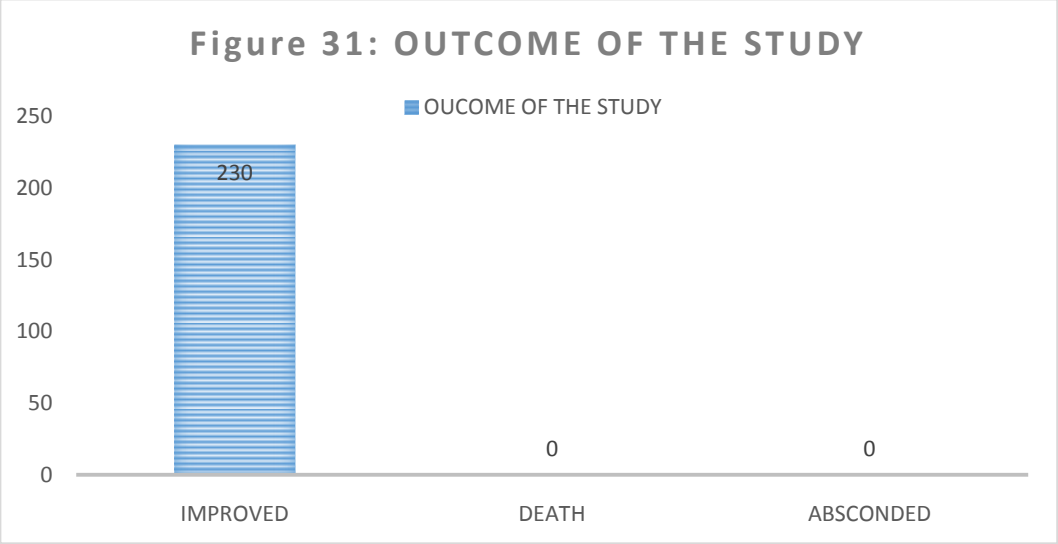


Figure 31: OUTCOME OF THE STUDY
OUCOME OF THE STUDY
250
200
150
100
50
0
230
0
0
IMPROVED
DEATH
ABSCONDED

6.0. Discussion:

Acute febrile illness is mostly caused by viruses followed by bacterial agents and parasites. The clinical spectrum though helps to suspect a particular disease, it needs confirmation in order to institute appropriate treatment, supportive care, prevention and containment. To achieve all these, clinical microbiologists play a pivotal role. The description on the clinico microbiological study of AUFI are given in the ensuing paragraphs.

6.1. Gender wise distribution:

Among the 230 febrile cases, males constituted 119 (51.74%) and 111 (48.3%) were females. There was no significant gender preponderance observed in the present study, in contrary to the study conducted by Gopalakrishnan S et al. [20], where male predominance was noted. By and large, males suffer various infective causes of AUFI probably due to their occupational nature, travel and some extent their behaviour.

6.2. Agewise distribution:

In the present study, a large number of cases belonged to the age group of 35-44 years and contribute about 47.83% . This was similar to the study, conducted by Abilash KP et al [11] and Rani RV et al. [19], in which the most common age group affected were below 40 years, with male to female ratio of 2:1 and constituted 59% of the study subjects. The variations in the occurrence of age group are mostly influenced by the location of the hospital, users and the socio demographic status of the population.

6.3. Monthwise distribution:

On analysis in relation to month, the number of cases enrolled were maximum from September to December and also in the month of January, due to favourable breeding sites following rainfull. This was similar to the study conducted by Rani RV et al. [19], where large number of cases were seen in the month of December (36). In a study by Shelke YP et al. [5] 62.22% cases were seen in the month of September and August. The seasonal variation vary from region to region, continent to continent, in view of geophysical causes and influence of climate changes.

6.4. Time of presentation of fever:

In the present study, the time of presentation of fever was mostly on the fourth day (14.9%) and sixth day (14%). In a study by Shelke YP et al. [5], the patients visited the hospital mostly on the 5th day of fever (18.88%) and 8th day of fever (25.18%). Health seeking behaviour is variably influenced by education, awareness, social status, income and previous experience.

6.5. Distribution of symptoms and age groups:

The analysis of symptoms in relation to age group was done and the most common symptom was myalgia in the age group 35-44 years. In the age groups of 15-34 and ≥45 years, headache and chills were common. This was similar to the study conducted by Abilash KP et al [11] and Shelke YP et al. [5], where the commonest presenting symptom were myalgia and headache. In general, the symptoms are

influenced by infective agents, immune response, pre-existing health status, behaviour of the individuals, etc.

6.6. Etiological causes:

In our study, the predominant cause was Dengue (56.2%) among the cases of AUFI, followed by Enteric fever- 29.3%, Malaria - 5.53%, , Scrub typhus – 2.97%, Chikungunya – 2.55%, Typhoid– 2.17% and Leptospirosis – 2.13%. The various studies analysed are given in Table 37, which reveals that the spectrum of AUFI vary from place to place in view of the environment, vectors and their behaviour.

Table 37: Correlation of various studies conducted on AUFI cases:

Studies done	Year	Dengue	Enteric fever	Malaria	Scrub typhus	Lepto spirosis	Chikungunya
Present study	2018	56.2%	30.6%	5.53%	2.97%	2.13%	2.55%
Shelke YP et al [5]	2017	17.4%	4%	12%	47%	2%	NE
Abhilash KP et al. [11]	2016	30.6%	3.7%	10.4%	35.9%	NE	NE
Rani RVet al. [19]	2015 –2016	27%	4%	2%	1%	NE	NE
Singh R et al. [35]	2013	71.2%	8.1%	12.8%	6.0%	NE	NE
Gopalakrishnan S et al. [20]	2010 - 2012	10.4%	20.59 %	33%	NE	6.2%	NE
Phuong HL et al. [34]	2010	19%	NE	NE	NE	NE	NE

Chrispal A et al. [13]	2008	7.0%	8.0%	17.1%	47.5%	3.0%	NE

*NE – NOT EVALUATED

6.7. Clinicomicrobiological aspects of Dengue:

Among the 132 dengue cases, male predominance was observed. Maximum number of cases were enrolled in the month of November. The most common presentation seen in dengue was myalgia, headache and chills. The cases which were positive were detected using Dengue IgM ELISA and NS1 ELISA. Among the 56.2% dengue cases, the positivity observed were Dengue IgM ELISA - 19.13% and Dengue NS1 antigen ELISA- 38.3% and both were positive in 13.04%. Our study results were similar to study conducted by Solanke et al. in 2015 [107] and Shelke YP et al. in 2016 [5] in which positivity observed for Dengue NS1 antigen ELISA was 71.6% and 72.34%, and Dengue IgM ELISA was 28.4% and 27.65%. In India, dengue positivity ranged between 8% and 71% among AUFI cases [108,20].

6.8. Clinicomicrobiological aspects of Enteric fever:

Enteric fever was the second most common cause of pyrexia, which accounted for 30.6% of cases. In cases of pyrexia, enteric fever accounted for 3% cases in a study conducted by Rani et al [19], in 2016. Enteric fever was the most common bacterial infection among southern Asian population [6], and accounted for a tenth of pyrexia cases in a study conducted in north India [12]. Many studies showed that Enteric fever was the aetiological agent in 8%–20% among the affected pyrexia cases in India [109].

Cases of Enteric fever were documented all over the year with no gender predominance. Features like myalgia, headache, nausea/vomiting, coated tongue, low total count were seen which was similar to other studies [5,113]. Five (6.9%) cases were positive by blood culture and the isolates were sensitive to both Chloramphenicol and Fluoroquinolones.

6.9. Clinicomicrobiological aspects of Malaria:

The third most common cause in the present study was Malaria (5.53%). Maximum number of cases visited the hospital in the month of November. Both male and female genders were equally affected. The positivity by peripheral smear was 92.3% and Immunochromatographic test detecting HRP2 and pLDH was 76.9%. Singh et al. [35] conducted a study on AUFI and had reported malaria (12.8%) as the second commonest cause among AUFI cases. In the study conducted by Rani et al. [19] and Abhilash KP et al. [11], Malaria was reported as the third common cause in fever cases in about 2% and 4.6% respectively. In a study conducted by Joshi R et al. [12], non malarial acute undifferentiated fever was observed in 39.9% cases.

6.10. Clinicomicrobiological aspects of Scrub typhus:

Scrub typhus was found in 7 (2.97%) febrile cases in contrary to the study conducted by Shelke YP et al. [5] where, the predominant cause of fever was scrub typhus (47%). The cases were maximum in the month of November and in the age group ≥ 45 years. Among them 6(2.56%) patients with scrub typhus presented with eschar on the body. The lesion was present in lower extremities and lower abdominal parts of body and was well circumscribed with delineated margin. Sinha et al.

conducted a study in 2014 [35] with 42 positive cases of scrub typhus, but eschar was absent among the 42 cases. In a study conducted by Shelke YP et al. in 2016 [5] and by Jung et al. in 2015 [110], the positivity showed was 47% and 56.6% respectively.

6.11. Clinicomicrobiological aspects of Chikungunya:

The positivity of Chikungunya cases in our study was 6 (2.55%), which was in contrast with the study conducted in 2012 by Ray P et al. [111], where the positivity was around 25.37%. Maximum cases were enrolled in the month of October with no gender preference. The cases mostly presented with myalgia and arthralgia. The positivity observed in rapid diagnostic test was 7(99.9%).

6.12. Clinicomicrobiological aspects of Leptospirosis:

In our study, Leptospirosis was diagnosed in 2.2% of pyrexia cases. This was similar to the study done by Shelke YP et al. in 2016 [5], where the positivity observed was 2%. Joshi R et al. [12] conducted a study, in which 3 out of 11 clinically suspected cases turned to be positive. 3% positivity was observed in the study carried out by Chrispal A et al. in 2008 [13]. The cases were seen more during the month of January with no gender preference. In the study conducted by Gopalakrishnan S et al., cases were seen more during the months of October and November, due to floods [20]. Features like myalgia, jaundice, abdominal pain were common. The positivity by Dark field microscopy and Lepto IgM ELISA was 2(40%) and 5(99.9%) The mortality rate observed in the study by Chrispal A et al [13] was 8.3%, while no mortality was seen in our study.

6.13. Co-infection:

The occurrence of co-infection is not uncommon in tropical countries due to factors like poor sanitation, over crowding, low socioeconomic status and poor immune status. The rate of co-infection between the Dengue and Typhoid cases seen in our study was 5(1.9%). The rate was higher i.e., 7.8% and 12.4% in a study conducted at North-India in 2013 by Sharma Y et al [112] and Parker TM et al [113] during the period of 2005-2006 in Egypt respectively. In a study conducted in 2013 at Northwest Ethiopia, co-infection between Malaria and Enteric fever was found to be 6.5% [114]; co-infection rate was 35% in males while, it was 37% in females, according to a study conducted by Odikamnoro et al [115] in Ebonyi State at 2017.

Table 39: Correlation of studies conducted on AUFI cases Co-infection positivity

Study done by	Country	Year	Diseases involved	Co-infection rate.
Sharma Y et al [112]	North-India	2013	Dengue and Typhoid	7.8%
Parker TM et al [113]	Egypt	2005-2006	Dengue and Typhoid	12.4%
Birhanie M et al [114]	Northwest Ethiopia	2014	Malaria and Enteric fever	6.5%
Odikamnoro et al [115]	Ebonyi State	2017	Malaria and Typhoid	35% in males and 37% in females

Microbiologists must exercise laboratory skills and with the clinical history and clinical course along with the interaction with the treating doctor have to make a diagnosis of dual/ mixed/ concurrent infections. The microbiologists play a major role towards diagnosis, treatment and prevention.

7.0 Summary

Acute febrile illness contributes to high morbidity and occasional mortality. Many a times, the diagnosis are suspected and attempts are made to make it probable and occasionally confirm the disease. As AUFI is of short duration, it does not receive the attention it deserves. Since the acute febrile illness is multi-faceted, an attempt has been made to study the clinicomicrobiological aspects of infective causes of AUFI.

- This cross sectional observational study was conducted in the Microbiology Department of Trichy SRM Medical College Hospital and Research Centre, from January to December 2018, after an approval from Institutional Ethics Committee.
- Out of the 500 hospitalised patients during the study period, only 230 patients satisfied the inclusion criteria and were included in our study. The socio-demographic, clinical, haematological and microbiological details were collected by adopting good clinical and laboratory practice. The data were entered in Microsoft Excel Spreadsheet and analysed using SPSS software 22.
- The cases included 119 males and 111 females. The hospital stay varied from 5 – 10 days and the median hospital stay was 5 days. The AUFI were more during the post monsoon and winter season.
- The spectrum of clinical profile observed in our study were in the order of Dengue, Enteric fever, Malaria, Typhoid followed by Scrub typhus, Leptospirosis and Chikungunya in 132 (56.2%), 67 (29.13%), 13 (5.53%), 7 (2.97%), 6 (2.55%), 5(2.17%) and 5 (2.13%) respectively. 5 among them had dual infection and suffered from Dengue and Enteric fever. Irrespective of the

gender, patients start attending the hospital from the fourth day of fever. The mean affected age was 40.9 years. The presentation of fever was maximum on the fourth day and sixth day of fever.

- The presenting features were more once the duration of disease increases, even though the commonest were myalgia, headache and chills.
- None of the patients had any complications and they were discharged within a period of 5 – 10 days.
- This study helped to find out the common causes of AUFI in this region. However, sizable number of cases might have suffered from infections due to other causes which needs further exploration.
- This clinicomicrobiological study on infective causes of acute febrile illness has brought out the social, legal, ethical, educational and professional responsibilities of clinical microbiologists.

8.0. Conclusion

In this study on "A CLINICOMICROBIOLOGICAL STUDY ON INFECTIVE CAUSES OF ACUTE FEBRILE ILLNESS" carried out over a period of consecutive 12 months, a total of 230 adult patients fulfilled the inclusion criteria. The conclusion arrived are given below:

- The overall occurrence of AUFI was independent of age group and gender, however it was observed significantly more in those above the age group of 35 years.
- The mean affected age was 40.9 years and the mean affected age for the males and females were 39.8 and 42.5 years respectively.
- AUFI was significantly more ($p<0.05$) among males and observed more during post monsoon and winter seasons (September to January).
- Irrespective of the gender, more number of patients start attending the hospital from the fourth day of fever.The median duration of the illness for males was 4^{th} day and for females was 7^{th} and 8^{th} day.
- The commonest symptom noticed among these cases, irrespective of gender were in the order of myalgia, headache and chills in 97.4, 88.9 and 85.9 % respectively. When analysing these symptoms in relation to age group, myalgia was seen more among the age group 35-44, whereas headache was noticed more among the age group 15-34 and 45 and above. Chills was noticed equally among all the age groups. The clinical course was uneventful and there were no mortalities among the 230 cases studied. The median hospital stay was 5 days and all of them recovered.

- The distribution of disease among the 230 cases were in the order of Dengue, Enteric fever, Malaria, Typhoid followed by Scrub typhus, Leptospirosis and Chikungunya in 132 (56.2%), 67 (29.13%), 13 (5.53%), 7 (2.97%), 6 (2.55%), 5(2.17%) and 5 (2.13%) respectively. 5 of the 230 (2.2%) had dual infection and suffered from Dengue and Enteric fever.
- The Syndromic approach for AUFIduring the study period revealed that Dengue and Enteric fever were prevalent all over the year and hence these two illness are most likely endemic in this study area.
- Other illneses included under this category such as Malaria, Scrub typhus, Leptospirosis and Chikungunya were seen occasionally and not all the months of the year.
- Of the 13 cases of Malaria, Peripheral smear studies helped to diagnose Malaria in 12 and 13 in Rapid Diagnostic test. Serology of these 13 cases did not interfere with other microbiological investigations carried out to diagnose the rest of the AUFI.
- Dark field microscopy of plasma assisted to identify Leptospirosis in 3 of the 5 diagnosed cases and IgM ELISA was helpful in diagnosing 5 cases.
- Dengue was diagnosed by NS1 antigen ELISA in 88 of the 132 cases and by IgM ELISA in the rest. 30 cases were found to be positive for NS1 antigen ELISA as well as IgM ELISA, while followed up, during their stay at hospital.
- *Salmonella typhi* was isolated only from 5 of the cases during the study and the organisms were sensitive to commonly used antimicrobials for *S. typhi* such as Chloramphenicol, Ciprofloxacin, Cefotaxime, Ceftriaxone and Cefepime. Diagnosis of Enteric fever was entertained based on cinical laboratory and

serology. Four fold rise in tire was demonstrated in 3 of the 15 cases by Widal test.

- Scrub typhus was diagnosed in 7 cases and Chikungunya in 6 cases by Rapid Diagnostic Test (Immunochromatographic test).
- All of the 230 cases considered for the study, recovered fully without any complications.

Strength of the study:

- A good standardised laboratory was available to carry out the study and good laboratory practices were maintained throughout the study.
- Standardised commercial kits were purchased for laboratory works
- Clinical studies and microbiological studies with regard to isolation and confirmation was monitored by Senior assistant professor and guide, and controversies were settled by Professor and Head of the Department.
- Clinical correlation was performed individually for all the cases of pyrexia and interpreted in consultation with Professor and Head of the Department of Microbiology.

Limitations of the study:

- The study is limited to the hospitalised Acute febrile illness cases in a single tertiary care hospital serving rural low income population.
- The study is limited to 6 diseases grouped under syndromic approach of AUFI.
- AUFI cases treated in the Paediatrics and Outpatient Department were not included and hence the true burden of AUFI could not be brought out.

- In our study, we could not carry out PCR. With the use of PCR, we could have detected more causative agent of AUFI.
- The microbiological studies were limited to 6 causes considered under Acute febrile illness and hence other causative agents were not attempted.

Future study:

- ✓ The clinical spectrum of AUFI needs second line of investigations to find out the emerging and re-emerging infective causes, if it is not included under the first 6. Region based study on AUFI is suggested to find out the pattern and prevalence of the disease causing AUFI, so as to plan for prevention and appropriate measures for containment.
- ✓ Microbiologists involved in AUFI have to work with Public health Department to contribute for policy making and design measures towards prevention and disease containment.

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