Research Article

A Cross-Sectional Three-Year Retrospective Survey on Acute Febrile Illness in Chennai.

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ABSTRACT

Background: Acute Febrile Illness (AFI), marked by nonspecific symptoms such as fever, myalgia, and arthralgia, is difficult to diagnose due to overlapping causes like Dengue, Chikungunya, Scrub typhus, and Leptospirosis. Limited diagnostic capacity often leads to delayed or inappropriate treatment.

Aim: This study retrospectively evaluated AFI cases referred to tertiary care centers in Chennai from 2021 to 2023 to determine etiological distribution, seasonal trends, and demographic patterns.

Methods: A total of 7,530 serum samples were subjected to ELISA-based assays targeting Dengue (NS1/IgM), Chikungunya (IgM), Scrub typhus (IgM), and Leptospirosis (IgM). Data were analyzed using ANOVA with post hoc tests to identify significant differences across years and diseases.

Results: Dengue was the predominant AFI cause in all three years (17.5% in 2021, 18.7% in 2022, and 15.9% in 2023) followed by Scrub typhus (2.8–3.9%) and Chikungunya (0.4–1.7%). Leptospirosis occurred in 0.15% (2021) and 0.29% (2022), but was absent in 2023. Notably, most cases remained undiagnosed: 77.9% in 2021, 76.6% in 2022, and 83.0% in 2023. Seasonal clustering was observed: Dengue peaked between September and December, while Scrub typhus and Leptospirosis displayed sporadic seasonal patterns.

Conclusion: AFI in Chennai is mainly driven by Dengue, with additional contributions from Scrub typhus and Chikungunya. The lack of Leptospirosis cases in 2023 may reflect temporal variation or referral bias. The high proportion of undiagnosed cases underscores diagnostic gaps. Strengthened point-of-care testing and syndromic management are essential for improving early detection, targeted treatment, and preventive efforts.

Keywords: *Acute Febrile Illness, Dengue, Chikungunya, Scrub Typhus, Leptospirosis, Chennai epidemiology.*

1. INTRODUCTION

Acute febrile illness (AFI), frequently referred to as a short febrile illness (SFI) or acute fever (AF) is generally referred to as a fever that persists for 14 days. Globally, medical practitioners have not yet reached a consensus on the exact definition of acute febrile illness due to ambiguity in the clinical manifestations of various AFI aetiologies [1]. This might involve an epidemic outbreak or pandemics of a larger proportion, especially in developing countries, accentuated by seasonal fluctuations and epidemic factors.

The etiology of AFI is multifactorial as bacteria, viruses, protozoa, rickettsia, and spirochaete contribute to the occurrence of diseases such as Leptospirosis, Chikungunya, Influenza, Rickettsial fevers, malaria, and scrub typhus. This panorama holds good for India and Southeast Asian countries. Based on the signs and severity of the illness, AFI is broadly divided into three subtypes:

Diagnosed AFI, Undiagnosed AFI, and Non-Malarial AFI [2, 3]. The disease is characterized by nonspecific symptoms such as headache, nausea, vomiting, diarrhea, body rash, myalgia, and arthralgia [4]. Moreover, due to varied etiological factors, endemicity, and pathological manifestations, the diagnosis of Acute febrile illness could be strenuous [5, 6]. Though the symptoms of acute febrile illness are nonspecific, a rational treatment strategy is required to prevent mortality. Also, to treat severe acute infections with precision and efficacy and to prevent the spread of antibiotic resistance in India, affordable, sensitive, and precise diagnostic techniques are essential. In addition, the difficulties in interpreting results based on diagnostic procedures include false positives due to previously encountered infections and cross-reactivity in serological tests. This necessitates more awareness of the strengths and limitations of diagnostic tests in India for proper therapeutic intervention [7].

According to previous case studies in India, patients who were hospitalized and diagnosed with malaria (8-17%), dengue (16-19%), scrub typhus (10-47%), Leptospira spp. (2-7%), Chikungunya (2-6%), and Salmonella spp. have been reported with acute fever without any localizing features [8, 9].

This study aims to determine the etiological distribution of acute febrile illness (AFI) in Chennai between 2021 and 2023, focusing on Dengue, Chikungunya, Scrub typhus, and Leptospirosis. It further sought to analyze seasonal and demographic patterns, including age- and gender-specific prevalence, and to assess the diagnostic yield of ELISA-based tests while highlighting the proportion of undiagnosed cases. By integrating epidemiological insights, the study intended to support clinicians in early suspicion, accurate diagnosis, and rational treatment of AFI, while also informing public health preparedness through identification of peak transmission periods and high-risk groups.

2.0 METHODS

2.1 Experimental Design and Sample Collection

A cross-sectional retrospective study was conducted across tertiary care centers in Chennai. The the facility to test the referred serum samples was provided by DHR/ICMR State Level Viral Research and Diagnostic Laboratory at KIPMR, Chennai. The study was approved by Human Institutional Ethical Committee. The current study was performed in accordance to relevant guidelines and regulations. Informed consent was acquired from all the participants of the study and/or their legal guardians.

2.2 Case definition

By the inclusion criteria, clinical specimens referred from individuals reported to have an undifferentiated fever that persisted for 14 days with or without rashes and body temperature ≥ 37.5 °C were analyzed in our study. The patients whose specific symptoms lasted 2 - 7 days were related to acute febrile illness (headache, malaise, abdominal pain, rigors, myalgia, and arthralgia) that can be detected as stated by WHO were involved in the study. Patients having additional symptoms unrelated to AFI and localized infections (e.g., pneumonia, urinary tract infections) met the criteria for exclusion from the study.

The samples that satisfied the symptoms associated with AFI were collected from 2021-23 and were classified based on IgM positivity for diagnosing diseases such as Dengue, Chikungunya, Scrub typhus, and Leptospirosis. A total of 7530 samples were collected from 2021 to 2023. The clinical specimens collected every year were analyzed with commercially available ELISA kits.

2.3 Sample Processing and Storage

The blood samples (3ml) were collected from suspected cases along with informed consent from patients. If samples were taken from children, consent from the parents of infants or guardians was obtained. Patient details and symptoms such as headache, body rash, arthralgia, myalgia, fever, and duration of illness were filled in the proforma. The samples were labeled and stored at -20°C until further analysis.

2.4 Detection of Antigen and Antibody

Antibody and antigen detection in the clinical samples was performed using the ELISA technique. Detection of serum NS1 antigen was done using commercially available ELISA kits for Dengue (*Abbott Panbio NS1 capture ELISA).*

NIV Dengue IgM ELISA, NIV Chikungunya IgM ELISA, In Bios Scrub Typhus Detect IgM ELISA and Panbio Leptospira IgM ELISA were used to detect IgM antibodies. All the tests were carried out as per the manufacturer's instructions. All the assay plates were analyzed using a Multiskan FC Microplate Photometer from *Thermo Scientific*.

2.5 Statistical Analysis

Software from San Diego, California's Graph Pad Prism 5.01 was used to conduct the statistical analysis. All the quantitative variables were presented as the mean ± SEM. The data from multiple groups were compared using non-parametric Tukey's multiple comparison test, followed by ANOVA. The statistical interpretation for symptomatic analyses, age-wise distribution with grouped variables, was compared between the groups using Two-way ANOVA using the Bonferroni test. Statistical significance was denoted at $P<0.05$, $P<0.01$ and $P<0.001$

3.0 RESULTS

A total of 2510, 1703, and 3317 samples were collected during 2021, 2022, and 2023, respectively, based on symptoms from individuals suspected to have an acute febrile illness and analysed. The patients screened for acute febrile illness had symptoms such as myalgia, arthralgia, fever persisting for more than 2 weeks, rashes, headache, diarrhoea, vomiting, rigors, malaise, undifferentiated rash, etc.

3.1 AFI Retrospective Survey and Diagnosis of Diseases

Out of 2510 samples, 438 were positive for Dengue, 42 were positive for Chikungunya, 71 were positive for Scrub typhus and 4 were positive for Leptospirosis in 2021 analyses (Table I). Subsequently, in 2022, among 1703 samples, 319 were NS1 / IgM positive for Dengue, 9 were IgM positive for Chikungunya disease, 66 samples were Scrub typhus positive and 5 were positive for Leptospirosis. Furthermore, in 2023, out of 3317 samples, 529 cases were positive for Dengue, 147 for Chikungunya, 19 for Scrub Typhus and 0 for Leptospirosis, respectively (Table I). Importantly, a majority of cases remained undiagnosed (77.9% in 2021, 76.6% in 2022, 83.0% in 2023). The percentage of positive cases reported during 2021-23 for Dengue, Chikungunya, Scrub typhus , Leptospirosis and undiagnosed cases were presented in Table I, respectively.

Table 1 - Distribution of AFI cases by etiological diagnosis and year (2021–2023)

Name of the disease diagnosed	Total number of detected cases			% Positives		
	2021	2022	2023	2021	2022	2023
Dengue positives	438	319	529	17.45	18.73	15.94
Chikungunya positives	42	9	14	1.67	0.52	0.42
Scrub Typhus positives	71	66	19	2.82	3.87	0.57
Leptospirosis positives	4	5	0	0.15	0.29	0
Undiagnosed illness	1955	1304	2755	77.9	76.6	83
Total AFI positives	2510	1703	3317			

Table I shows the total AFI positives, number of cases detected with Dengue, Chikungunya, Scrub typhus and Leptospirosis and the percentage of positive cases detected from 2021 – 2023.

The total AFI positives detected in 2021 and 2023 were significantly ($P<0.001$) high as compared to the diagnosed cases of Dengue, Chikungunya, Scrub typhus, and Leptospirosis, respectively. Similarly, the reported number of total AFI positives in 2022 was significantly high as compared to the detected number of Dengue ($P<0.01$), Chikungunya ($P<0.001$), Scrub typhus ($P<0.01$), and Leptospirosis ($P<0.001$) positives (Fig. 2).

3.2 Symptomatic Analyses of Dengue, Chikungunya, Scrub Typhus and Leptospirosis

Patients who tested positive for Dengue predominantly had symptoms such as severe headache, high fever, fatigue, joint pain, skin hemorrhages, arthralgia, abdominal pain, swollen glands, retro-orbital pain, anorexia, nausea, vomiting, and rashes. The reported symptoms of headache, myalgia, and diarrhea were significant ($P<0.05$) in Dengue positives as compared to total AFI positives. On the other hand, Scrub typhus-positive patients exhibited characteristic symptoms such as eschar, maculopapular rashes, and lymph node enlargement along with AFI symptoms.

Symptoms such as fever (P<0.05), headache (P<0.01), myalgia (P<0.001), vomiting (P<0.01), diarrhea (P<0.01), and arthralgia (P<0.05) exhibited significant changes in Scrub typhus positives when compared to AFI positives. In addition, AFI patients who were tested Chikungunya positive developed joint pain and papular rashes. Significant discrepancies in fever (P<0.01), headache (P<0.01), myalgia (P<0.001), vomiting (P<0.01), diarrhea (P<0.001), and arthralgia (P<0.05) were noticed in Chikungunya positives and Leptospirosis positives as compared to total AFI positives. Furthermore, symptoms such as breathlessness, rashes, headache, and myalgia were observed predominantly in Leptospirosis-positive patients. (Fig. 1).

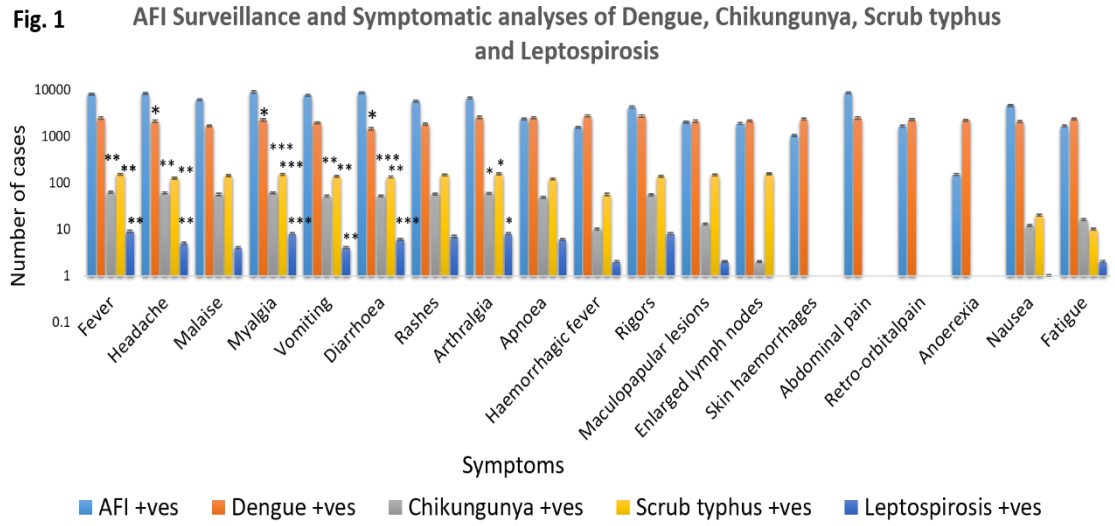


Fig. 1 AFI surveillance and comparative study of symptoms between the detected number of positive cases of Chikungunya, Scrub typhus and Leptospirosis by ELISA (2021 – 2023)

* denotes significance at P<0.05 as compared to AFI+ves

** denotes significance at P<0.01 as compared to AFI+ves

*** denotes significance at P<0.001 as compared to AFI+ves

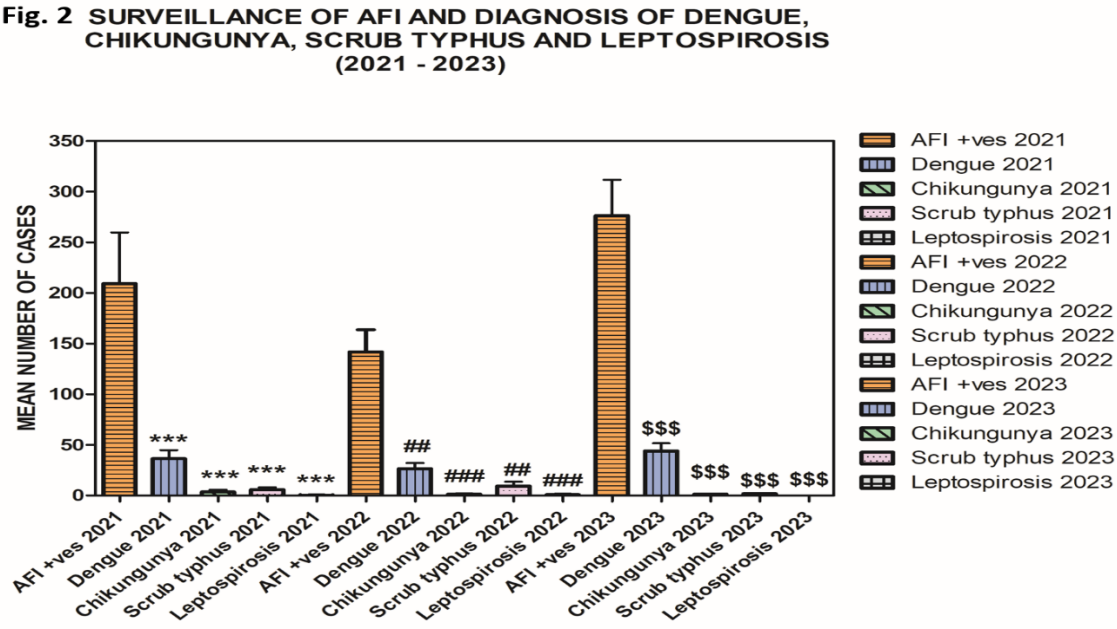


Fig. 2 Acute febrile illness and Diagnosis of Dengue, Chikungunya, Scrub typhus and Leptospirosis by ELISA in 2021-23 representing the number of reported cases

*** denotes significance at P<0.001 as compared to total AFI+ves 2021.

denotes significance at P<0.01 as compared to AFI+ves 2022

denotes significance at P<0.001 as compared to AFI+ves 2022

$$$ denotes significance at P<0.001 as compared to AFI+ves 2023

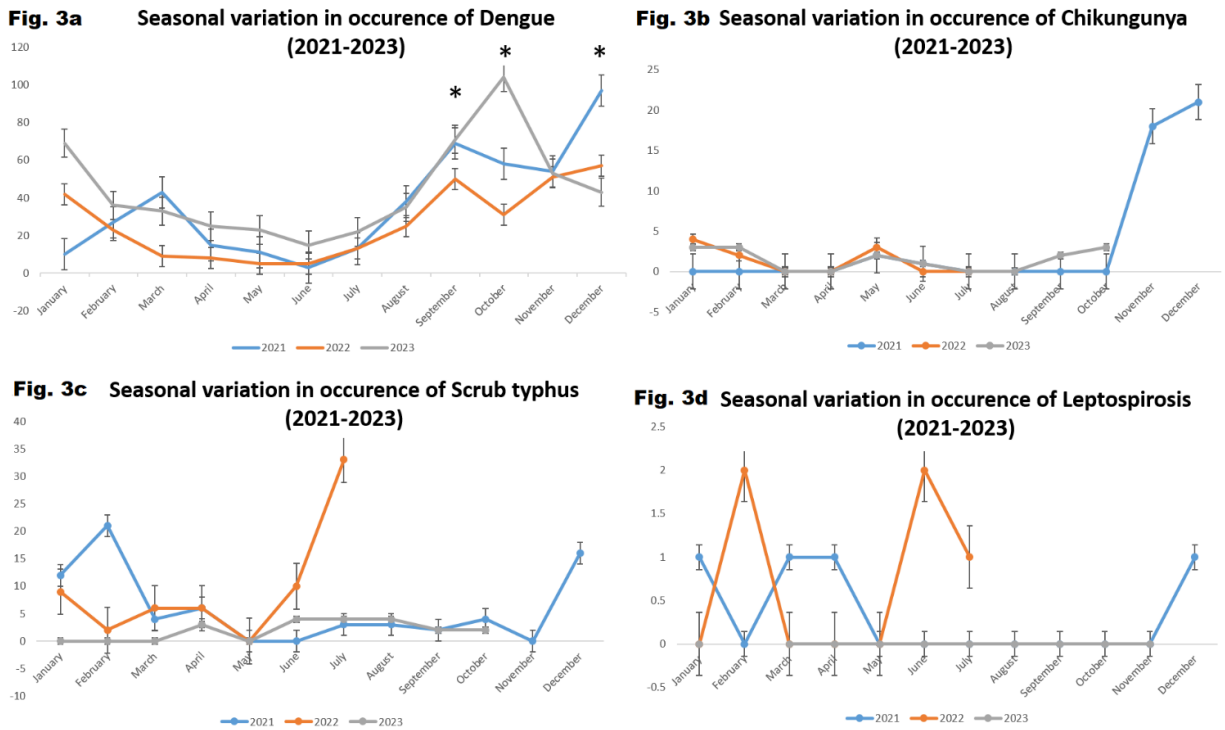


Fig. 3a. Seasonal variation in occurrence of Dengue; *denotes significance for Dengue positives at P<0.05 as compared to June month. 3b. Seasonal variation in occurrence of Chikungunya, 3c. Seasonal variation in occurrence of Scrub typhus; 3d. Seasonal variation in the occurrence of Leptospirosis

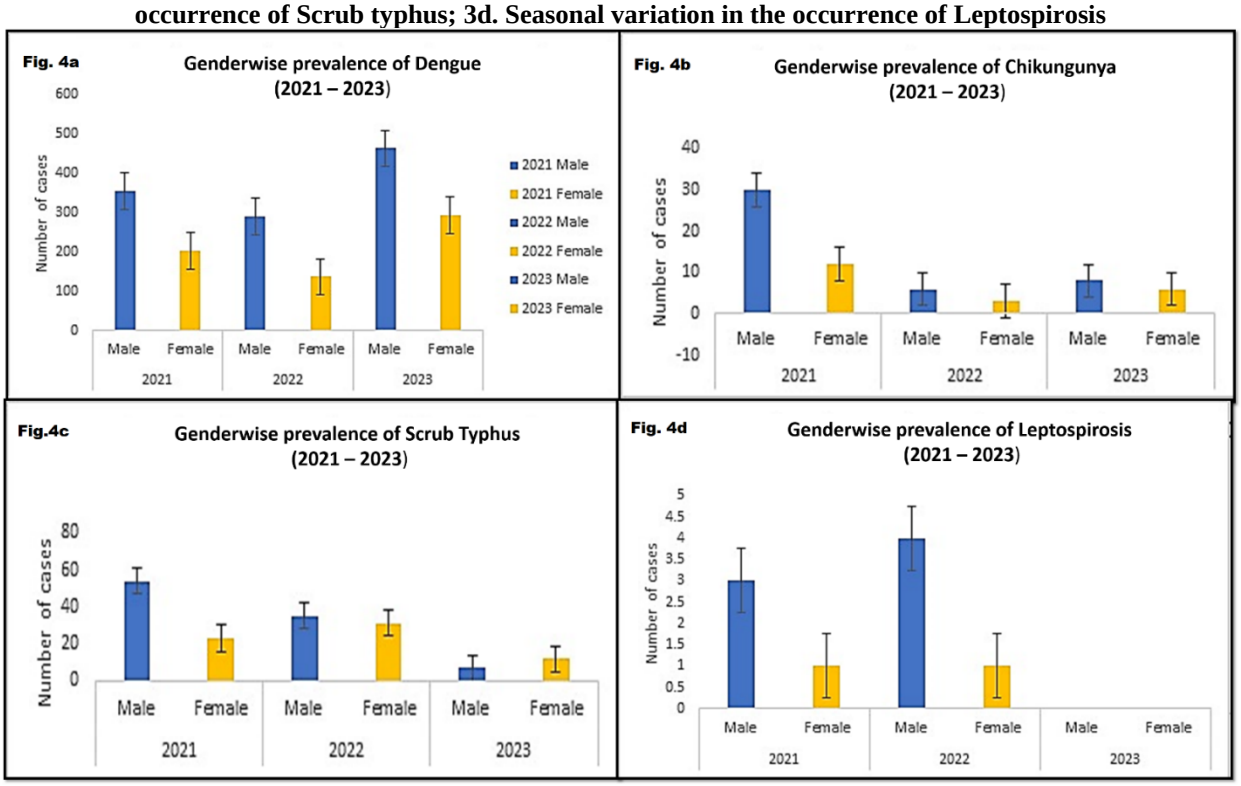


Fig. 4a. Gender-wise prevalence of Dengue (2021 – 2023); 4b. Gender-wise prevalence of Chikungunya (2021- 2023) 4c. Gender-wise prevalence of Scrub typhus (2021 – 2023); 4d. Gender-wise prevalence of Leptospirosis (2021 – 2023)

3.3 Seasonal Variation in the occurrence of Dengue, Chikungunya, Scrub Typhus and Leptospirosis

The frequency of acute febrile illness has been reported throughout the year (2021-23) as AFI positives, as shown in Fig. 3. However, the

overall seasonality of AFI could be influenced by the high prevalence of Dengue positives reported from September to December and the increased incidence of Chikungunya positives during November and December. In 2021, the highest Dengue incidence was reported in December (22.1%). Besides, Dengue cases were also reported during September (15.7%), October (13.2%), and November (12.3%), respectively. Similarly, in 2022, increased Dengue cases were reported during December (17.8%). Similarly, Dengue cases were also identified in November (15.9%) and September (15.6%) in 2022. Whereas in 2023 analyses, a greater number of Dengue cases were detected during October (19.6%), followed by September (13.4%) and January (13.0%), respectively (Fig. 3a). Chikungunya positives were increasingly reported during December (50%) and November (42.8%) in 2021. Whereas in 2022, positive cases were only detected during January (44.4%), February (22.2%), and May (33.3%). In 2023, a minimal number of Chikungunya positives were recorded during January (21.4%), February (21.4%), and October (21.4%) (Fig. 3b). In addition, Scrub typhus positives in 2021 were highest during February (29.5%) followed by December (22.5%) and the maximal incidence of Scrub typhus among all three years (2021-23) was recorded in July (50%) in 2022. Besides, a few Scrub typhus positives were only detected in 2023 from April to October (15-21%) (Fig. 3c). In 2021, the incidence of Leptospirosis positives was predominant during January (25%), March (25%), and April (25%). Subsequently, in 2022, diagnosis for Leptospirosis exhibited more number of positives during February (40%) and June (40%), and in 2023, no positive cases were detected (Fig. 3d). Overall, the total number of cases recorded every month was statistically compared with each year for all the diseases respectively. As a result, the total number of Dengue positives recorded during September, October, and December significantly increased ($P<0.05$) as compared to June month. On the other hand, no significant changes were observed for diseases namely Chikungunya, Scrub typhus, and Leptospirosis.

3.4 Gender and Age-wise Prevalence

A total of 2510 samples from AFI cases were referred in 2021. Of these, 1491 were male and 1019 were female. In 2022, among 1,703 AFI positives, 999 were male and 704 were female. In 2023, out of 3317 AFI positives, 1799 were male and 1518 were female. The age-wise distribution of total AFI positives in 2021, 2022, and 2023 is detailed in Table II. The highest incidence of acute febrile illness was observed in the 36 – 55 years and 19-35 years age groups in all three years. Additionally, during the study period (2021-23), an average of 4.5% of children aged 0- 2 years and 35.66% of children aged 3- 12 years were affected by Dengue in the pediatric age group.

Table 2- Age-wise distribution of AFI-positive cases from 2021 to 2023.

Age group	Total AFI positives (2021)	Total AFI positives (2022)	Total AFI positives (2023)
0-5 yrs	135	134	136
6-12 yrs	286	234	439
13-18 yrs	286	233	522
19-35 yrs	967	632	1350
36-55 yrs	579	347	662
Above 55 yrs	257	123	228

Table II shows Age-wise distribution of total AFI positives detected from 2021- 2023.

Collaboratively, it was evident during the study (2021-23) that males (64.2%) were more affected by Dengue compared to females (35.7%), as shown in Fig. 4a. Overall, during the same period, it was observed that young adults aged 20 to 29 years (92.6%) were most susceptible to developing Dengue. Additionally, adults in age groups between 3 to 12 years (35.6%), 13 to 19 years (34.6%), 30 to 39 years (29.6%), and 40 to 49 years (18.6%) were also affected, as illustrated in Fig. 5a. The occurrence of Dengue among the 13 to 19-year age group significantly increased ($P<0.05$) in 2023 compared to 2021.

Similar to Dengue, the incidence of infection was higher in males (65%) with Chikungunya illness than in females (34.9%), as observed in the present study from 2021-23, as shown in Fig. 4b.

The age groups most affected by Chikungunya were 30 to 39 years, followed by 20 to 29 years (2021-23). In 2022, patients affected were from the 30 to 39 years and 20 to 29 years age groups. Subsequently, in 2023 analyses, the 40 to 49 years and 30 to 39 years age groups were most affected, Fig. 5b.

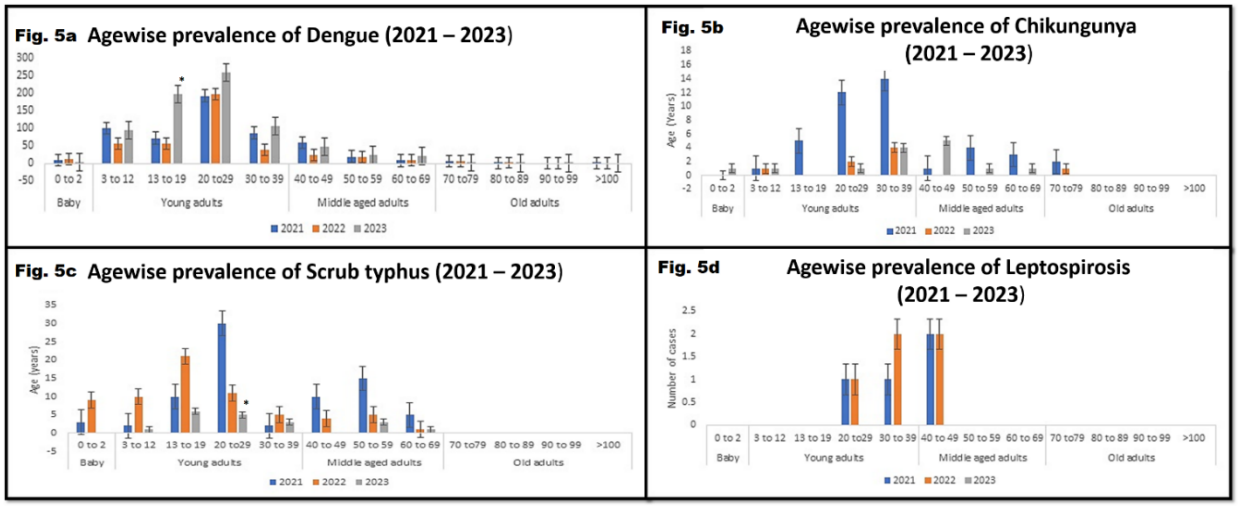


Fig. 5a. Age wise distribution of Dengue (2021 – 2023); 5b. Age-wise prevalence of Chikungunya (2021 – 2023); 5c. Age-wise distribution of Scrub typhus (2021 – 2023); 5d. Age-wise distribution of Leptospirosis (2021 – 2023)

* denotes significance at P<0.05 as compared to total AFI+ves 2021.

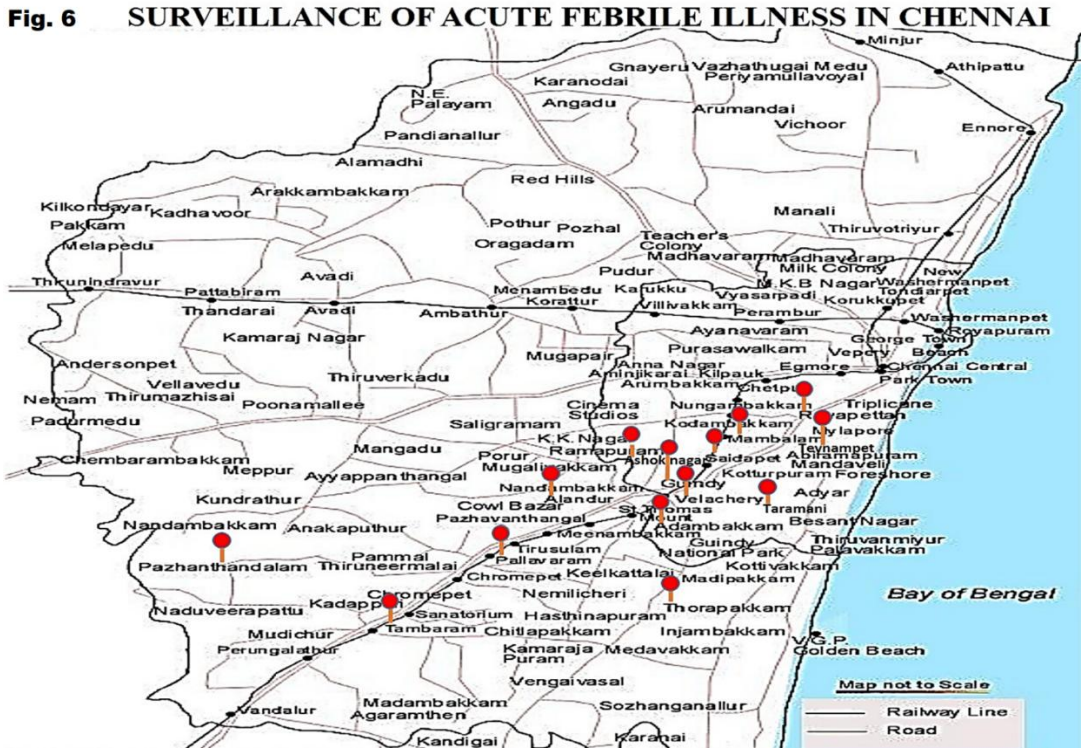


Fig. 6. Surveillance of Acute Febrile Illness in Chennai

In 2021 (70.1%) and 2022 (53.0%) analyses, males were more highly affected by Scrub typhus illness than females (2021 – 29.9%, 2022 – 46.9%). In 2023, diagnosis of Scrub typhus showed a higher prevalence in females (63.1%) than in males (36.8%) (Fig. 4c). Also, the highest incidence of Scrub typhus was recorded in patients belonging to the age group 20 to 29 followed by 50 to 59 in 2021. In 2022, 13 to 19-year-old patients were increasingly affected by Scrub typhus, followed by 3 to 12 and 20 to 29-year-olds. In 2023 analyses, highly affected Scrub typhus positives belonged to age groups 13 to 19, followed by 20 to 29 (Fig. 5c). The occurrence of Scrub typhus infection in 20-29

years was significantly ($P<0.05$) high in 2021 as compared to 2023.

Collectively, from 2021-23, more males (51.6%) were infected with leptospirosis as compared to females (40%) (Fig. 4d). Also, an increased number of leptospirosis-positive cases were from age groups such as 40 to 49 followed by 30 to 39 and 20 to 29 (Fig. 5d). The current retrospective survey of acute febrile illness in various tertiary health care centers and hospitals had shown the areas that were widely affected with AFI in Chennai. AFI mapping, as shown in Fig. 6, shows the geographical distribution of acute febrile illness in Chennai within the population.

4 DISCUSSION

Acute febrile illness, with frequent atand challenging clinical presentation, is often associated with symptoms such as headache, muscle pain, cough, nausea, dyspnea, joint pain, rashes, retro-orbital pain, diarrhea, hepatomegaly, splenomegaly, icterus, etc [9] which are non-specific leading to faulty clinical diagnosis and ineffective treatment. Therefore effective treatment for AFI demands a syndromic approach with well-tested laboratory tests for common organisms, preventing undue usage of antibiotics [10]. According to studies, most arthropod-borne infections, such as Dengue, Malaria, Chikungunya, and Scrub-Typhus were predominantly reported in India, contributing to the etiology of acute febrile illness [8, 11].

A study in September 2022 in Panchkula, Haryana, reported that out of 58 patients, 45 (77.5%) were diagnosed with Dengue, and two with Chikungunya, with no positives for Zika, West Nile, Scrub typhus, or Leptospira [12]. Additionally, in 2016, six IgM Chikungunya positives and 151 IgM-specific dengue positives out of 200 AFI positives were reported in Mumbai [13]. Furthermore, Scrub typhus affects multiple regions in India, including Tamil Nadu, Andhra Pradesh, Karnataka, Kerala, Himachal Pradesh, Uttaranchal, Jammu and Kashmir, Meghalaya, Assam, Nagaland, West Bengal, Bihar, and Maharashtra [14]. Nonetheless, since 2010, an increase in reported Scrub typhus cases has led to the development of better confirmatory diagnostic tests, such as Scrub typhus ELISA and RT-PCR, enabling improved validation [15]. Moreover, Leptospirosis, associated with acute febrile illness, is a significant cause of morbidity and mortality in India, often linked to bleeding manifestations like thrombocytopenia and coagulation abnormalities [17]. A recent study involving 169 clinically tested samples from rural and urban patients of Central India with acute febrile illnesses found 35 (20.7%) positive cases of Scrub typhus and one positive case of Leptospirosis [18].

The dynamics of mosquito populations and disease transmission primarily depend on factors like temperature and precipitation. The number of cases each year can vary due to seasonality, as lower temperatures and increased rainfall create favorable habitats for larvae and pupae [19, 20]. The study shows a high prevalence of Dengue infection in Chennai during the northeast monsoon (September–January), Chikungunya in November and December, and scrub typhus in August and February. Leptospirosis is mainly transmitted during monsoon seasons, with the highest cases occurring in June and July [21, 22]. According to earlier reports from a tertiary care center in South India, the incidence of Dengue and Leptospirosis was higher in males than in females [23], which aligns with the findings of the current study. Additionally, recent reports on Scrub typhus from a tertiary healthcare center in Western Odisha indicated a higher percentage of infected males than females, while some previous reports from West Bengal showed more infected females [24, 25]. However, in the present study, males were more affected by Scrub typhus in the studies conducted in 2021 and 2022. In contrast, the 2023 diagnosis of Scrub typhus revealed more infected females. Another diagnostic study of Chikungunya from a tertiary healthcare center reported higher susceptibility in males than females [26], which correlates with the findings of this study.

Moreover, the present retrospective survey (2021-23) showed a maximum number of Dengue cases from the age group 20 - 29 years. This could be corroborated by previous findings with 21 to 30 years largely affected by Dengue [27]. In addition, patients collectively affected with Chikungunya from 2021-23 were

predominantly from the age groups 20-29 and 30-39 years, respectively. This could be correlated to a cross-sectional study conducted in Chennai where patients affected with Chikungunya were predominantly from the age group, 15-44 years [28]. Also, reports on diagnosis for scrub typhus showed that patients aged 1 to 20 years were primarily affected [29]. Whereas in the present study, patients under the age groups 13-19 and 20-29 years were positive for scrub typhus. In the present study, the 40-49 years age group, followed by the 30-39 years were highly prone to Leptospirosis. On the contrary, previous reports from Maharashtra, India, revealed most Leptospirosis cases from the 20-35 age group [30].

The results acquired from the current retrospective survey of acute febrile illness (2021-23) unveiled significantly more AFI-positives when compared to individually detected Dengue, Chikungunya, Scrub typhus, and Leptospirosis positives. The number of Dengue-positive cases detected in 2023 was substantially higher than those reported in 2021. Likewise, throughout the study period (2021-23), the detected number of Dengue cases was substantially higher than the Chikungunya, Scrub typhus, and Leptospirosis positives, respectively. The clinical significance of the current study includes effective and timely diagnosis of the most commonly suspected etiological agents of AFI, such as Dengue, Chikungunya, Scrub typhus, and Leptospirosis, from the clinical specimens received from tertiary care centers in Chennai throughout the year (2021-23). Also, the combined use of NS1 antigen and IgM detection in Dengue demonstrates high diagnostic accuracy.

Noteworthily, the epidemiology of acute febrile illness in Chennai demonstrates clear seasonal and demographic patterns, influenced by the region's tropical climate and vector ecology. Dengue cases were consistently highest during the northeast monsoon season (September–December), with peaks recorded in December 2021 (22.1%), December 2022 (17.8%), and October 2023 (19.6%). This aligns with the increased breeding of Aedes aegypti mosquitoes during post-monsoon months. Chikungunya cases showed a more sporadic distribution, with notable clustering in November and December 2021 (42–50%) and smaller peaks in early 2022 and 2023, suggesting localized outbreaks. Scrub typhus was most prevalent in February 2021 and July 2022, reflecting its association with cooler or post-rainy periods when mite populations thrive. Leptospirosis cases, although rare in this study, were reported in the early monsoon months of 2021 and 2022, consistent with its known link to waterlogging and rodent exposure, but no cases were detected in 2023.

Together, these findings indicate that Chennai's AFI burden is strongly shaped by monsoon-driven seasonality, vector ecology, and sociodemographic exposure risks, underscoring the importance of targeted seasonal surveillance and public health preparedness in the region

The National Centre for Vector-borne Disease Control reported a cyclic increase, with the incidence increasing from 6039 in 2021 to 9121 in 2023 for Dengue and 3654 in 2021 to 4805 in 2023 for Chikungunya in Tamil Nadu [31]. Scrub typhus recurrence has also increased in various regions of India, with significant outbreaks in Meghalaya, Himachal Pradesh, Nagaland, Tamil Nadu, and Puducherry [32]. Leptospirosis outbreaks in India have increased over the past three decades, with the southern region having the highest positivity rate at 25.6% [33]. A recent meta-analysis from AIIMS, Rishikesh, highlights the clinical significance of scrub typhus-leptospirosis co-infection in India. It also highlights the need for improved diagnostic techniques, increased clinical awareness, and focused research efforts to address the new infectious disease threat [34].

Also, COVID-19's high prevalence in developing tropical/sub-tropical nations led to increased co-infections and elevated mortality risk. Delays in diagnosing and managing acute febrile illness (AFI) have been caused by overlapping symptoms, resource allocation, and inadequate infection control measures [35]. The variation observed in yearly sample numbers (2021: 2,510 → 2022: 1,703 → 2023: 3,317 in the current retrospective study could be attributed to COVID-19 disruption, seasonal and demographic patterns. Despite India's disease

surveillance program [32], frequent monitoring for acute febrile illnesses is crucial for accurate disease incidence, timely treatment, and appropriate control measures.

The current retrospective study has important limitations. First, the analysis relied on serum samples referred from tertiary care centers, which may introduce referral bias, as these cases are likely to represent more severe illnesses than those managed at primary care level. Second, only ELISA-based serological assays were used for diagnosis; molecular methods such as RT-PCR or the microscopic agglutination test (MAT) for Leptospirosis were not performed. This restriction may have led to false positives due to antibody persistence or cross-reactivity, and false negatives in early infections where antibodies had not yet developed. Third, the large proportion of undiagnosed AFI cases (>75%) highlights gaps in the diagnostic panel, suggesting that other viral, bacterial, or parasitic etiologies were not captured. Fourth, the absence of a control population or community-based surveillance limits the ability to estimate true prevalence and risk factors at the population level. Fifth, the year-to-year variation in case numbers (notably the drop in 2022 and rise in 2023) could have been influenced by external factors such as COVID-19-related healthcare disruptions, which were not accounted for in this study. Finally, environmental and vector-related parameters, such as rainfall and mosquito density, were not integrated, which could have strengthened interpretation of seasonal trends. Despite these limitations, the study provides valuable baseline epidemiological data on AFI in Chennai and underscores the need for expanded diagnostics, prospective surveillance, and integrated clinical-epidemiological studies in the future.

However, in Chennai, acute febrile illness (AFI) is a significant clinical challenge, with local epidemiology highlighting Dengue, Chikungunya, etc as major causes. A frequent survey on acute febrile illness will help clinicians and epidemiologists to raise awareness of these potential aetiologies and the use of appropriate diagnostic and management strategies. The current study conducted by the diagnostic research laboratory under the VRDL network will liaise with the public health authorities to combat threatening viral infections.

However, the practical recommendations advise clinicians to adopt a syndromic approach supported by confirmatory ELISA or molecular testing, given the overlapping symptoms of Dengue, Chikungunya, Scrub typhus, and Leptospirosis. Early recognition of warning signs such as persistent fever, rash, eschar, or severe myalgia is critical, especially during monsoon and post-monsoon months when AFI peaks. Since most AFI cases remain undiagnosed and may be viral in origin, unnecessary broad-spectrum antibiotic use should be minimized to prevent antimicrobial resistance. Strengthening seasonal preparedness through vigilance during high-risk months and aligning clinical practice with local epidemiological patterns will enhance case detection and patient outcomes.

5. CONCLUSION

Acute Febrile Illness remains a major public health challenge in Chennai, with Dengue identified as the leading cause during 2021–2023, followed by Scrub typhus, Chikungunya, and sporadic Leptospirosis cases. The absence of Leptospirosis in 2023 emphasizes the need to consider seasonal and epidemiological variability. Importantly, more than 75% of AFI cases remained undiagnosed, underscoring limitations of the current diagnostic framework.

This study highlights the necessity of expanding beyond ELISA-based testing to include molecular diagnostics such as RT-PCR, which would reduce false positives and improve detection of co-infections. Referral bias from tertiary centers and the lack of control populations remain limitations, but the findings still provide valuable insights into Chennai's AFI burden.

From a clinical perspective, the study supports a syndromic approach combined with laboratory confirmation, enabling healthcare providers to differentiate between vector-borne and bacterial etiologies, thus minimizing inappropriate antibiotic use. Public health authorities should strengthen surveillance during peak monsoon months, improve diagnostic coverage, and

enhance clinician awareness to mitigate morbidity and mortality associated with AFI.

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CONFLICT OF INTEREST

The authors wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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DATA AVAILABILITY STATEMENT

The data will be available upon request from the corresponding author (kaveriraj1967@gmail.com)

ETHICAL INFORMATION

The Institutional Ethics Committee (Ref No. 010/ KIPMR/2021/Date: 14.02.2021), King Institute of Preventive Medicine and Research, waived ethical approval in view of the retrospective nature of the study and the fact that all the procedures performed were part of routine care.

AUTHOR CONTRIBUTIONS STATEMENT

All authors have accepted responsibility for the entire content of this manuscript and approved its submission. **Vasanth Mohandass (VM)**: Methodology, Validation, Formal analysis, Data collection, Data curation. **Ravi Kiran (RK)**: Methodology, Validation, Formal analysis, Data collection, Data curation. **Roshan Raj (RR)**: Methodology, Data collection, **Lakshmipriya Navaneethakrishnan (LN)**: Data collection, Formal analysis, **Divya Sankaramourthy (DS)**: Data curation, Validation, Formal analysis, writing: original draft, review & editing**,** Visualization**. Yamini Gopi (YG)**: Methodology, Data collection, Data curation, Formal analysis. **Santhosh Rajakumar (SR):** Methodology, Data collection, Data curation, Formal analysis. **Bhavyaa Lakshmi Sole (BLS):** Methodology, Data collection, Data curation, Formal analysis. **Anupama Cherayi Padinakara (ACP):** writing, review & editing, supervision. **Kavery Krishnasamy (K.K):** Conceptualization, Methodology, Validation**,** Formal analysis, Investigation, Resources, writing: review & editing**:** supervision, Project administration.

ABBREVIATIONS

AFI - Acute febrile illness
AUFI - Acute undifferentiated febrile illness
CHIKV - Chikungunya virus
ELISA - Enzyme-linked Immunosorbent Assay
IgM - Immunoglobulin M
KIPMR - King Institute of Preventive Medicine & Research
RT-PCR - Real-time Polymerase Chain Reaction
VRDL - Viral Research and Diagnostic Laboratory
WHO - World Health Organisation

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