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Original Research Article

Microbiological Profile of Dengue, Malaria and Chikungunya among Patients Presenting with Acute Febrile Illness in a Tertiary Care Hospital

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ABSTRACT

Background: Acute febrile illness (AFI) is a leading cause of hospital attendance in India, and dengue, malaria and chikungunya share overlapping clinical features and seasonality. Accurate laboratory-based etiological profiling is essential for appropriate management and for guiding public health action.

Aim and objectives: To determine the microbiological profile of dengue, malaria and chikungunya among patients with AFI, and to study their demographic, seasonal, clinical, laboratory and outcome characteristics, including co-infections.

Materials and methods: This hospital-based, cross-sectional observational study was conducted in the Department of Microbiology of a tertiary care hospital from July 2025 to June 2026. Four hundred consecutive patients with AFI were enrolled. Dengue was diagnosed by NS1 antigen and IgM/IgG capture ELISA, malaria by Giemsa-stained peripheral smear and rapid diagnostic test (RDT), and chikungunya by IgM capture ELISA. Other etiologies were evaluated by blood culture, Widal test, scrub typhus and leptospira IgM ELISA, and urine culture. Data were analysed using the chi-square test, Fisher exact test and ANOVA; $p < 0.05$ was significant.

Results: The mean age was 32.8 ± 16.4 years and 59.5% were males. A specific vector-borne etiology was identified in 202 patients (50.5%). Dengue was the commonest (125; 31.3%), followed by malaria (52; 13.0%) and chikungunya (41; 10.3%); co-infections occurred in 15 patients (3.8%). Positivity peaked during the monsoon and post-monsoon months (September–October). Dengue positivity differed significantly by season ($p < 0.001$), and malaria was significantly more frequent in males ($p = 0.033$). NS1 and IgM positivity in dengue were 62.4% and 56.8%, respectively, with NS1 positivity declining and IgM rising after day 5 of illness. *Plasmodium vivax* accounted for 63.5% of malaria cases and *P. falciparum* for 30.8%; RDT showed a higher yield (94.2%) than peripheral smear (88.5%). Arthralgia (87.8%) characterised chikungunya, chills and splenomegaly characterised malaria, and thrombocytopenia (78.4%) characterised dengue. Overall mortality was 1.5%.

Conclusion: Dengue, malaria and chikungunya together accounted for half of all AFI cases, with significant seasonal clustering and a non-negligible burden of co-infection. A combined diagnostic approach (NS1 plus IgM for dengue; smear plus RDT for malaria) and early, simultaneous testing during the monsoon season are recommended.

Keywords: *Acute febrile illness; dengue; malaria; chikungunya; NS1 antigen; co-infection; tertiary care hospital; India.*

INTRODUCTION

Acute febrile illness (AFI) is one of the commonest presenting complaints in the outpatient and inpatient services of tertiary care hospitals in tropical and subtropical countries, and its differential diagnosis is exceptionally wide[1,2]. Because the early clinical picture of many infections consists of non-specific fever, headache, myalgia and malaise, vector-borne diseases such as

dengue, malaria and chikungunya are difficult to separate on clinical grounds from enteric fever, rickettsial infections, leptospirosis, viral hepatitis and bacterial sepsis[3,4]. A delay in etiological diagnosis leads to empirical and often unnecessary antimicrobial use, and to missed opportunities for timely supportive care in conditions where early fluid management or antimalarial treatment is life saving.

Dengue is caused by four antigenically distinct serotypes of dengue virus (DENV-1 to DENV-4), a flavivirus transmitted mainly by *Aedes aegypti* and *Ae. albopictus*. It is the most important arboviral disease of humans, with an estimated 390 million infections annually, of which about 96 million are clinically apparent[5]. The clinical spectrum ranges from a self-limiting febrile illness to dengue haemorrhagic fever, plasma leakage and shock[6]. India is hyperendemic for all four serotypes, and the disease has spread from large cities to small towns and rural areas, with recurrent outbreaks every monsoon season[7,8]. The 2017 national serosurvey showed that nearly half of the Indian population aged five years and above carried IgG antibodies indicating past infection[9], and a systematic review has documented wide regional variation in dengue prevalence[10]. Despite this, dengue remains substantially under-reported through routine surveillance[11], and its incidence is closely linked to rainfall, temperature and humidity[12].

Malaria continues to be a major public health problem, and India contributes the largest share of malaria cases in the WHO South-East Asia Region. Both *Plasmodium vivax* and *P. falciparum* are transmitted in India, and the long-standing burden of the disease has been well described[13,14]. National mortality surveys suggest that deaths due to malaria are considerably higher than those captured by routine reporting[15]. Although the national incidence has fallen over the last decade, tertiary hospitals still receive complicated cases, including severe disease caused by *P. vivax*, which was once regarded as benign[16].

Chikungunya virus is an *Aedes*-transmitted alphavirus (family Togaviridae) that has re-emerged globally over the last two decades[17]. After an absence of about three decades, it re-appeared in India in 2005–2006 and caused extensive outbreaks across several states[18–20], with an excess mortality being reported during the Ahmedabad epidemic[21]. The hallmark of the illness is fever with severe, often incapacitating, polyarthralgia, and a multi-centric Indian study has confirmed its importance as a cause of febrile illness presenting to hospitals[22].

Dengue and chikungunya share the same *Aedes* vectors, and all three infections peak during or soon after the monsoon, so that dual or even triple infections are possible. Dengue–chikungunya co-infections have been described from Delhi and West Bengal[23,24], and concurrent dengue and malaria has been reported from several Indian centres[25,26]. Co-infection can modify the clinical presentation, delay recognition of the second pathogen and influence the outcome.

Laboratory confirmation is therefore indispensable. Dengue can be detected by NS1 antigen during the early febrile phase and by IgM antibodies thereafter[27,28]; malaria is diagnosed by microscopy of blood smears supported by rapid diagnostic tests (RDTs)[29,30]; and chikungunya by IgM serology or molecular detection depending on the day of illness[31]. Locally generated, hospital-level data on the relative burden, seasonality, clinical correlates and diagnostic yield of these three infections are essential for designing rational diagnostic algorithms and for informing vector-borne disease control activities. The present study was undertaken to provide such a microbiological profile from a tertiary care hospital.

Aim

To study the microbiological profile of dengue, malaria and chikungunya among patients presenting with acute febrile illness in a tertiary care hospital.

Objectives

To determine the proportion of dengue, malaria and chikungunya among patients with AFI. To study the age, sex and seasonal distribution of these infections. To compare the clinical and laboratory features of the three infections and to identify co-infections. To evaluate the diagnostic markers (NS1, IgM, IgG; smear and RDT) and the clinical outcome of patients.

MATERIAL AND METHODS

Study design, setting and duration

This was a hospital-based, cross-sectional observational study conducted in the Department of Microbiology in collaboration with the Departments of General Medicine and Paediatrics of a tertiary care teaching hospital over a period of 12 months, from July 2025 to June 2026. Patients were recruited from the outpatient department, emergency services and inpatient wards.

Study population and case definition

AFI was defined as fever of 38 °C (100.4 °F) or higher, of duration not exceeding 14 days, with or without other symptoms, in a patient of any age. Consecutive patients meeting this definition were enrolled after obtaining informed consent (and assent/parental consent for children).

Inclusion criteria: patients of all ages and both sexes with AFI for whom a blood sample could be obtained within 14 days of onset of fever.

Exclusion criteria: (i) fever lasting more than 14 days; (ii) fever with an obvious localised focus of infection at presentation (e.g. clinical pneumonia, skin and soft-tissue infection); (iii) patients who had already received antimalarial treatment before sampling; and (iv) patients or guardians who declined consent.

Sample size and sampling

The sample size was calculated using the formula $n = Z^2pq/d^2$[32], taking an expected prevalence (p) of 50% for any vector-borne etiology (as no local estimate was available), an absolute precision (d) of 5% and a 95% confidence level, which gave a minimum of 384 patients. This was rounded off to 400. Consecutive sampling was followed.

Clinical data collection

A pre-designed, pre-tested pro forma was used to record demographic details, duration and day of illness at sampling, presenting symptoms, examination findings (rash, hepatosplenomegaly, bleeding manifestations, signs of plasma leakage), place of residence, and history of travel. Dengue was classified into dengue without warning signs, dengue with warning signs and severe dengue according to the revised WHO classification[33] and the national guidelines[34]. Malaria was categorised as uncomplicated or severe according to national and WHO criteria[35,36]. Outcome (discharge, ICU admission, death) and duration of hospital stay were noted.

Sample collection and processing

Three to five millilitres of venous blood (1–2 mL in children) was collected under aseptic precautions. An EDTA sample was used for the complete blood count (automated haematology analyser), peripheral smear and malaria RDT; a plain sample was allowed to clot and serum was separated and tested immediately or stored at −20 °C until batch testing for serological assays and liver function tests. Blood culture bottles were inoculated at the bedside in patients with suspected bacteraemia.

Laboratory diagnosis

Dengue. All samples were tested for dengue NS1 antigen by a commercial ELISA[37,38], and for dengue-specific IgM and IgG antibodies by capture ELISA, strictly as per the manufacturers' instructions. A case was labelled as dengue if NS1 antigen and/or IgM antibody was positive. IgG positivity was recorded separately as a marker of past or secondary infection.

Malaria. Thick and thin smears were prepared, stained with Giemsa (or Leishman) stain and examined under the oil-immersion objective by two independent microbiologists; a smear was reported negative only after examination of at least 100 oil-immersion fields. Species identification and parasite density were recorded. A bivalent antigen-detecting RDT (PfHRP-2/pan-pLDH or Pv-pLDH) was performed simultaneously on every sample[29,30]. A case was labelled as malaria if either test was positive, with clinical response to antimalarial therapy supporting the diagnosis in smear-negative/RDT-positive patients.

Chikungunya. Serum was tested for chikungunya-specific IgM antibodies by antibody-capture (MAC) ELISA[31]. Samples obtained within the first four days of illness with a negative IgM result were re-tested on a convalescent sample where the patient was available. Molecular testing was not available during the study period.

Other etiologies. Patients negative for all three infections were evaluated for other causes of AFI: blood culture by the automated system with identification and susceptibility testing by standard methods, Widal tube agglutination (interpreted with caution because of its known limitations[39]), scrub typhus IgM ELISA (in line with national

rickettsial disease guidelines[40]), leptospira IgM ELISA, urine routine microscopy and culture, and hepatitis A/E IgM where clinically indicated.

Quality control

Positive and negative kit controls were included in each ELISA run, and plate validity criteria were applied as per the manufacturer. Smear staining quality was checked with each batch, and all positive and 10% of negative smears were cross-checked by a second observer. RDTs were stored and used according to the recommended temperature and reading time.

Ethical considerations

The study was approved by the Institutional Ethics Committee before enrolment. Written informed consent was obtained from all participants or their guardians, and confidentiality of the patient data was maintained. Positive results were communicated immediately to the treating physician, and notifiable infections were reported to the hospital epidemiology unit.

Statistical analysis

Data were entered in Microsoft Excel and analysed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Categorical variables are expressed as numbers and percentages, and continuous variables as mean ± standard deviation. The chi-square test or Fisher exact test was used to compare proportions, and one-way ANOVA was used to compare means among groups. A p value of less than 0.05 was considered statistically significant.

RESULTS

During the study period, 468 patients with fever were screened, of whom 68 were excluded (fever longer than 14 days, n = 29; localised focus of infection, n = 18; prior antimalarial treatment, n = 11; consent refused, n = 10). The remaining 400 patients constituted the study population.

Demographic profile

Of the 400 patients, 238 (59.5%) were males and 162 (40.5%) were females, giving a male-to-female ratio of 1.47:1. The age ranged from 1 to 82 years (mean 32.8 ± 16.4 years). The most commonly affected age group was 15–30 years (37.0%), followed by 31–45 years (26.5%) (Table 1).

Age group (years)	Male n (%)	Female n (%)	Total n (%)
0–14	31 (13.0)	21 (13.0)	52 (13.0)
15–30	91 (38.2)	57 (35.2)	148 (37.0)
31–45	62 (26.1)	44 (27.2)	106 (26.5)
46–60	38 (16.0)	26 (16.0)	64 (16.0)
>60	16 (6.7)	14 (8.6)	30 (7.5)
Total	**238 (100)**	**162 (100)**	**400 (100)**

Table 1: Age and sex distribution of patients with acute febrile illness (N = 400)

Percentages are column percentages.

Etiological profile

A specific etiology among the three target infections was established in 202 of the 400 patients (50.5%), whereas 198 (49.5%) were negative for dengue, malaria and chikungunya. Considering all patients with a given infection (including co-infections), dengue was detected in 125 (31.3%), malaria in 52 (13.0%) and chikungunya in 41 (10.3%). Mono-infections accounted for 187 patients (46.8%) and co-infections for 15 patients (3.8%; 7.4% of all positives). Dengue–chikungunya was the commonest co-infection pair (n = 7), followed by dengue–malaria (n = 5) (Table 2).

Etiology	n	% of total (N = 400)	% of positives (n = 202)
Mono-infections	187	46.8	92.6
Dengue only	112	28.0	55.4
Malaria only	44	11.0	21.8
Chikungunya only	31	7.8	15.3
Co-infections	15	3.8	7.4
Dengue + Malaria	5	1.3	2.5
Dengue + Chikungunya	7	1.8	3.5
Malaria + Chikungunya	2	0.5	1.0
Dengue + Malaria + Chikungunya	1	0.3	0.5
Total positive for ≥1 infection	202	50.5	100
Negative for all three	198	49.5	–
Total	**400**	**100**	**–**

Table 2: Distribution of dengue, malaria and chikungunya among patients with AFI

Overall (including co-infections): dengue 125 (31.3%), malaria 52 (13.0%), chikungunya 41 (10.3%).

Age and sex distribution

Dengue and malaria were most frequent in the 15–30 year age group, in which positivity for dengue was 35.1% and for malaria 14.2%. Chikungunya positivity increased with age, from 5.8% in children to 17.2% in the 46–60 year group, although the difference across age groups was not statistically significant (p = 0.19) (Table 3). Males predominated among dengue (33.6% vs 27.8%) and malaria cases (16.0% vs 8.6%), the latter difference being statistically significant (p = 0.033), whereas chikungunya was slightly more frequent in females (13.0% vs 8.4%; p = 0.14) (Table 4).

Age group (years)	Total	Dengue n (%)	Malaria n (%)	Chikungunya n (%)
0–14	52	14 (26.9)	6 (11.5)	3 (5.8)
15–30	148	52 (35.1)	21 (14.2)	11 (7.4)
31–45	106	35 (33.0)	15 (14.2)	12 (11.3)
46–60	64	17 (26.6)	7 (10.9)	11 (17.2)
>60	30	7 (23.3)	3 (10.0)	4 (13.3)
Total	400	125 (31.3)	52 (13.0)	41 (10.3)
p value (chi-square)	–	0.53	0.93	0.19

Table 3: Age-wise distribution of dengue, malaria and chikungunya

Percentages are row percentages (positivity within the age group). Co-infected patients are counted under each infection.

Sex	Total	Dengue n (%)	Malaria n (%)	Chikungunya n (%)
Male	238	80 (33.6)	38 (16.0)	20 (8.4)
Female	162	45 (27.8)	14 (8.6)	21 (13.0)
Total	400	125 (31.3)	52 (13.0)	41 (10.3)
p value (chi-square)	–	0.217	0.033*	0.140

Table 4: Sex-wise distribution of dengue, malaria and chikungunya

Statistically significant (p < 0.05)

Seasonal and monthly distribution

Cases were distributed throughout the year but clustered in the monsoon and post-monsoon months. Dengue positivity rose from July, peaked in September (27 cases) and remained high in October (24 cases), whereas malaria cases peaked slightly earlier, in July (9 cases). Chikungunya peaked in October (9 cases), following the dengue peak by about a month (Table 5, Figure 1). Dengue positivity was 13.9% in January–May, 35.1% in June–September and 41.0% in October–December ($p < 0.001$), whereas the seasonal variation in malaria ($p = 0.32$) and chikungunya ($p = 0.57$) was not statistically significant (Table 6).

Month	AFI patients	Dengue	Malaria	Chikungunya
January	18	3	2	2
February	16	2	1	1
March	20	2	2	2
April	22	3	3	1
May	25	4	4	2
June	30	6	6	3
July	46	14	9	4
August	56	21	8	5
September	62	27	7	8
October	54	24	5	9
November	30	12	3	3
December	21	7	2	1
Total	**400**	**125**	**52**	**41**

Table 5: Monthly distribution of AFI patients and positive cases

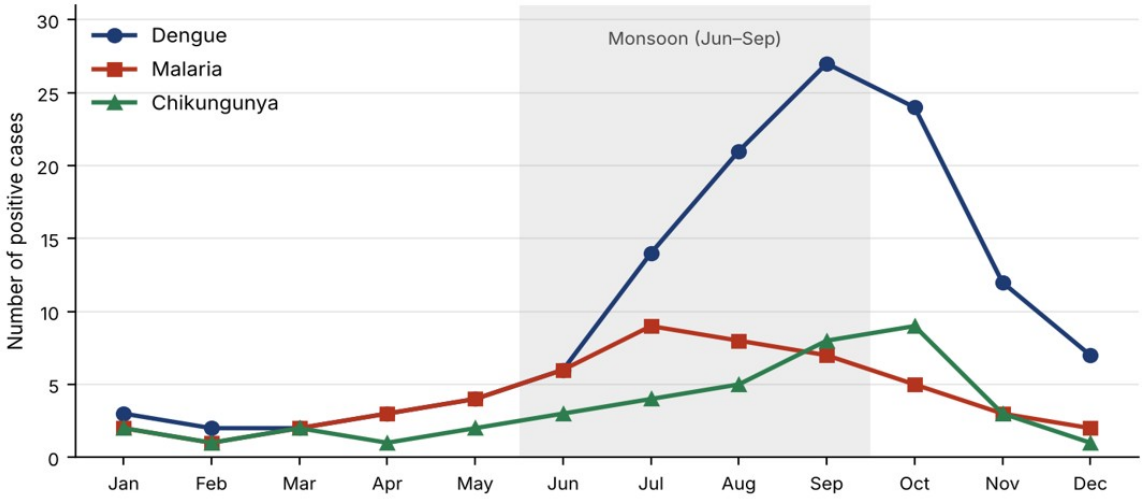


Figure 1: Monthly distribution of dengue, malaria and chikungunya positive cases

Season	AFI patients	Dengue n (%)	Malaria n (%)	Chikungunya n (%)
Winter–summer (Jan–May)	101	14 (13.9)	12 (11.9)	8 (7.9)
Monsoon (Jun–Sep)	194	68 (35.1)	30 (15.5)	20 (10.3)
Post-monsoon (Oct–Dec)	105	43 (41.0)	10 (9.5)	13 (12.4)
p value (chi-square)	–	<0.001*	0.32	0.57

Table 6: Season-wise positivity of dengue, malaria and chikungunya

Statistically significant ($p < 0.05$). Percentages are positivity within the season

Clinical features

Fever was universal. Headache and myalgia were common to all three infections, but distinct patterns emerged. Chills and rigors (90.4%) and splenomegaly (50.0%) were significantly more frequent in malaria; rash (23.2%) and bleeding manifestations (13.6%) in dengue; and arthralgia (87.8%) in chikungunya ($p < 0.001$ for each). Jaundice was more frequent in malaria (17.3%) than in dengue (4.8%) or chikungunya (2.4%) (Table 7).

Clinical feature	Dengue (n = 125) n (%)	Malaria (n = 52) n (%)	Chikungunya (n = 41) n (%)	p value
Headache	98 (78.4)	40 (76.9)	27 (65.9)	0.265
Myalgia	91 (72.8)	29 (55.8)	33 (80.5)	0.022*
Arthralgia	41 (32.8)	8 (15.4)	36 (87.8)	<0.001*
Chills and rigors	31 (24.8)	47 (90.4)	11 (26.8)	<0.001*
Rash	29 (23.2)	2 (3.8)	14 (34.1)	0.001*
Vomiting	54 (43.2)	21 (40.4)	9 (22.0)	0.050
Abdominal pain	38 (30.4)	12 (23.1)	5 (12.2)	0.063
Bleeding manifestations	17 (13.6)	2 (3.8)	1 (2.4)	0.031*
Splenomegaly	11 (8.8)	26 (50.0)	2 (4.9)	<0.001*
Jaundice	6 (4.8)	9 (17.3)	1 (2.4)	0.006*

Table 7: Comparison of clinical features among dengue, malaria and chikungunya

Statistically significant ($p < 0.05$; chi-square or Fisher exact test). Co-infected patients are included under each infection

Haematological and biochemical parameters

Platelet counts and total leucocyte counts were lowest in dengue, while haemoglobin was lowest in malaria. Serum aspartate aminotransferase (AST) was markedly raised in dengue compared with the other groups. All differences among the four groups were statistically significant ($p < 0.001$) (Table 8).

Parameter	Dengue (n = 125)	Malaria (n = 52)	Chikungunya (n = 41)	Negative group (n = 198)	p value
Haemoglobin (g/dL)	12.4 ± 1.8	10.6 ± 2.1	12.9 ± 1.7	12.6 ± 1.9	<0.001
Total leucocyte count (×10³/µL)	4.1 ± 1.9	6.8 ± 2.6	6.4 ± 2.2	9.2 ± 3.4	<0.001
Platelet count (×10³/µL)	92.4 ± 58.6	108.3 ± 62.1	214.7 ± 71.3	238.5 ± 84.6	<0.001
AST (U/L)	142 ± 96	78 ± 52	48 ± 27	41 ± 22	<0.001

Table 8: Haematological and biochemical parameters (mean ± SD)

One-way ANOVA. Co-infected patients are included under each infection.

Dengue: diagnostic markers and severity

Among the 125 dengue patients, NS1 antigen was positive in 78 (62.4%) and IgM antibody in 71 (56.8%). NS1 alone was positive in 54 (43.2%), IgM alone in 47 (37.6%) and both markers in 24 (19.2%) (Table 9), so that 43.2% of cases would have been missed had IgM alone been used and 37.6% had NS1 alone been used. IgG antibody was positive in 38 patients (30.4%). NS1 positivity declined progressively with the day of illness, from 78.8% within the first three days to 16.7% after the seventh day, whereas IgM positivity increased from 26.9% to 100% over the same period (Table 10).

Marker pattern	n	%
NS1 antigen positive (total)	78	62.4
IgM antibody positive (total)	71	56.8
NS1 positive, IgM negative	54	43.2
NS1 negative, IgM positive	47	37.6
NS1 and IgM both positive	24	19.2
IgG antibody positive	38	30.4

Table 9: Dengue diagnostic markers (n = 125)

Day of illness	Dengue cases	NS1 positive n (%)	IgM positive n (%)
≤3 days	52	41 (78.8)	14 (26.9)
4–5 days	41	28 (68.3)	28 (68.3)
6–7 days	20	7 (35.0)	17 (85.0)
>7 days	12	2 (16.7)	12 (100)
Total	**125**	**78 (62.4)**	**71 (56.8)**

Table 10: NS1 and IgM positivity by day of illness at sampling (dengue cases, n = 125)

According to the revised WHO classification, 79 patients (63.2%) had dengue without warning signs, 38 (30.4%) had dengue with warning signs and 8 (6.4%) had severe dengue (Table 11). Thrombocytopenia (platelet count < 150 × 10^3/µL) was present in 98 patients (78.4%), and 31 (24.8%) had counts below 50 × 10^3/µL (Table 12).

Category	n	%
Dengue without warning signs	79	63.2
Dengue with warning signs	38	30.4
Severe dengue	8	6.4
Total	**125**	**100**

Table 11: Severity of dengue according to the revised WHO classification (n = 125)

Platelet count (/µL)	n	%
≥150,000	27	21.6
100,000–149,999	38	30.4
50,000–99,999	29	23.2
<50,000	31	24.8
Total	**125**	**100**

Table 12: Platelet count distribution in dengue patients (n = 125)

Malaria: species and diagnostic methods

Of the 52 malaria cases, 33 (63.5%) were due to *P. vivax*, 16 (30.8%) due to *P. falciparum* and 3 (5.8%) were mixed infections (Table 13). Five patients (9.6%) had severe malaria: four with *P. falciparum* (25.0% of falciparum cases) and one with *P. vivax* (3.0%); the manifestations were severe anaemia (n = 2), cerebral malaria (n = 1), acute kidney injury (n = 1) and severe jaundice (n = 1). Peripheral smear was positive in 46 patients (88.5%) and RDT in 49 (94.2%); both were positive in 43 (82.7%), smear alone in 3 (5.8%) and RDT alone in 6 (11.5%) (Table 14).

Species	n	%	Severe malaria n (%)
Plasmodium vivax	33	63.5	1 (3.0)
Plasmodium falciparum	16	30.8	4 (25.0)
Mixed (*P. vivax* + *P. falciparum*)	3	5.8	0 (0)
Total	**52**	**100**	**5 (9.6)**

Table 13: Species distribution of malaria parasites (n = 52)

Percentages are rounded to one decimal place.

Result pattern	n	%
Peripheral smear positive (total)	46	88.5
RDT positive (total)	49	94.2
Both smear and RDT positive	43	82.7
Smear positive, RDT negative	3	5.8
Smear negative, RDT positive	6	11.5

Table 14: Diagnostic yield of peripheral smear and RDT in malaria (n = 52)

Composite reference standard: positivity on either test with clinical response to antimalarial treatment.

Chikungunya and co-infections

All 41 chikungunya cases were diagnosed by IgM ELISA, at a mean sampling day of 6.2 ± 2.1 of illness. Polyarthralgia was present in 36 (87.8%), mostly involving the small joints of the hands, wrists and ankles bilaterally (n = 29), and a maculopapular rash in 14 (34.1%). Of the 15 co-infected patients, 12 (80.0%) required admission, three needed intensive care and one patient with dengue–malaria co-infection died. Mean haemoglobin and platelet counts in the co-infected group were 10.9 ± 1.9 g/dL and 84.2 ± 49.5 × 10^3/µL, respectively, lower than in the corresponding mono-infections.

Other causes of AFI in the negative group

Among the 198 patients negative for the three target infections, an alternative etiology was identified in 154 (77.8%). Enteric fever (19.2%), viral upper respiratory/influenza-like illness (14.1%), bacterial sepsis with positive blood culture (12.1%), urinary tract infection (11.1%) and scrub typhus (10.6%) were the major diagnoses, while 44 patients (22.2%) remained undiagnosed and recovered spontaneously (Table 15).

Diagnosis	n	%
Enteric fever (blood culture/Widal)	38	19.2
Viral URTI/influenza-like illness	28	14.1
Bacterial sepsis (positive blood culture)	24	12.1
Urinary tract infection	22	11.1
Scrub typhus	21	10.6
Hepatitis A/E and other causes	12	6.1
Leptospirosis	9	4.5
No cause identified (self-limiting)	44	22.2
Total	**198**	**100**

Table 15: Etiology of AFI among patients negative for dengue, malaria and chikungunya (n = 198)

Clinical outcome

Overall, 214 patients (53.5%) required hospital admission and 21 (5.3%) needed intensive care. Six patients died (case fatality 1.5%): two with dengue, two with malaria (both *P. falciparum*), one with dengue–malaria co-infection and one in the negative group (bacterial sepsis). The proportion requiring admission, intensive care and the duration of stay were highest among the co-infected patients (Table 16).

Group	n	Admitted n (%)	ICU n (%)	Deaths n (%)	Hospital stay (days)*
Dengue only	112	71 (63.4)	8 (7.1)	2 (1.8)	5.4 ± 2.2
Malaria only	44	30 (68.2)	4 (9.1)	2 (4.5)	4.7 ± 1.8
Chikungunya only	31	13 (41.9)	1 (3.2)	0 (0)	4.2 ± 1.6
Co-infections	15	12 (80.0)	3 (20.0)	1 (6.7)	7.1 ± 2.9
Negative group	198	88 (44.4)	5 (2.5)	1 (0.5)	4.9 ± 2.4
Total	**400**	**214 (53.5)**	**21 (5.3)**	**6 (1.5)**	**–**

Table 16: Clinical outcome according to etiological group (N = 400)

Mean ± SD among admitted patients

DISCUSSION

AFI remains a diagnostic challenge in tertiary hospitals because a large number of treatable and potentially fatal infections present with a similar picture. In the present study, one of the three target vector-borne infections was documented in half of the patients (50.5%), with dengue (31.3%) well ahead of malaria (13.0%) and chikungunya (10.3%). This is in keeping with hospital-based Indian studies in which dengue, malaria, enteric fever, scrub typhus and leptospirosis together account for most AFI diagnoses, with the relative share of each depending on geography, season and the diagnostic tests available. The fact that another cause was identified in 77.8% of the negative group underlines that a negative vector-borne work-up should prompt evaluation for enteric fever, rickettsial disease, sepsis and urinary infection, rather than closure of the diagnostic process.

The predominance of young adults (15–45 years, 63.5%) and of males (59.5%) mirrors earlier reports from northern and southern India[41,42], and is usually attributed to greater outdoor and occupational exposure to vectors and to better health-care seeking by working-age men. The significant male excess among our malaria patients (p = 0.033) is consistent with the Indian experience, where occupational and migrant populations bear a disproportionate burden. The tendency of chikungunya to increase with age and to affect more women, although statistically non-significant here, has also been observed in Indian outbreaks and probably reflects both domestic exposure to peridomestic *Aedes* breeding and the greater symptomatic expression of arthralgia in older adults.

The sharp rise of dengue from July, with a peak in September–October, follows the monsoon rainfall that creates breeding sites for *Aedes* mosquitoes, and the dengue positivity in the monsoon and post-monsoon months was roughly 2.5 to 3 times higher than in the January–May period. Similar monsoon-linked patterns, tightly correlated with rainfall, temperature and humidity, have been reported across India and in the broader South-East Asian region[43]. The chikungunya peak in October, about four weeks after dengue, may reflect the same vector-driven dynamics and the lag in IgM detectability. In contrast, malaria showed a flatter distribution with a modest rise in July, in line with its dependence on *Anopheles* breeding in stagnant water and on local transmission foci.

The NS1 positivity of 62.4% and IgM positivity of 56.8% in our dengue patients are explained by the timing of sampling: NS1 antigen was detected in nearly four of every five patients within three days of fever but in only 16.7% after day seven, whereas IgM positivity rose to 100% after the first week. This reciprocal relationship has been described extensively, and the combined use of NS1 and IgM gives a markedly higher diagnostic yield than either test alone. In our series, 43.2% of cases were NS1-positive/IgM-negative and 37.6% IgM-positive/NS1-negative, so that relying on a single marker would have missed a substantial proportion. An IgG positivity of 30.4% points to a considerable fraction of secondary or past infections in this endemic setting, which is relevant because sequential infection with a heterologous serotype is the best-recognised risk factor for severe disease[44]. We found that 30.4% of patients had warning signs and 6.4% had severe dengue, a distribution compatible with the revised WHO classification, which was designed to identify patients who need closer observation more sensitively than the older haemorrhagic fever grading[45]. Severe

forms were described in the large Delhi epidemic in children[46], and the changing epidemiology of the disease in that city suggests a shift towards adults and more complicated presentations. Thrombocytopenia in 78.4% and the marked AST elevation in our patients parallel earlier Indian and international observations, in which low platelet counts and transaminitis were the most consistent laboratory correlates of dengue[47,48].

P. vivax accounted for 63.5% of malaria in this study, a proportion consistent with the predominance of vivax malaria in many Indian regions[49]. Although *P. falciparum* constituted only 30.8% of cases, it contributed four of the five severe cases and both malaria deaths, in agreement with its recognised virulence. Importantly, one severe case was due to *P. vivax*, which supports reports from Bikaner and other centres showing that vivax malaria can be complicated by severe anaemia, thrombocytopenia, jaundice and organ dysfunction[16]. The RDT detected 94.2% of cases against 88.5% for smear microscopy, and six cases were RDT-positive/smear-negative. Microscopy remains the reference method because it provides species and parasite density, but it depends on skilled personnel and parasite load, whereas RDTs perform well for falciparum malaria though they may remain positive after parasite clearance when HRP-2 is used. Running both tests together was thus clinically valuable. The lower platelet counts and haemoglobin seen in our malaria patients are well-documented features of the disease[50].

Fever with polyarthralgia (87.8%) of the small joints was the typical presentation, similar to the pattern described in multi-centric Indian data and in the 2006 outbreaks in India. The prominence of joint involvement distinguishes chikungunya from dengue even when both are co-circulating, and chikungunya is also notable for prolonged arthritic sequelae in a proportion of patients[51,52]. Because the viraemic phase is short, IgM-based diagnosis is appropriate only after the fourth to fifth day, while molecular methods are preferable earlier multiplex RT-PCR assays that detect dengue and chikungunya simultaneously have been validated in Indian settings and would increase the diagnostic yield in the early febrile phase[53]. The absence of molecular testing in our laboratory is therefore a limitation, and may have led to under-detection of early chikungunya.

Co-infections were present in 3.8% of all patients and in 7.4% of positive patients, and dengue–chikungunya was the commonest pair. Studies from Delhi and West Bengal have documented dual dengue–chikungunya infection and noted that its clinical features overlap with those of the mono-infections. Concurrent malaria and dengue has been described from Kerala, Odisha and other parts of India, often presenting with severe thrombocytopenia, anaemia and a more complicated course[54]; a regional review also notes this overlap[55]. Our co-infected patients had the highest admission (80.0%), ICU (20.0%) and mortality (6.7%) rates and the longest hospital stay, but the numbers are small and the findings must be interpreted cautiously. The practical message is that a positive result for one pathogen should not stop the work-up in a patient with atypical features, a severe course or a poor response to treatment.

Chills with rigors and splenomegaly strongly suggested malaria, rash and bleeding favoured dengue, and arthralgia pointed to chikungunya. However, each of these features was also present, in varying proportions, in the other infections and in the negative group, and no single feature was sufficiently specific to replace laboratory confirmation. This overlap has been repeatedly stressed in Indian AFI series. Clinical pointers can thus be used to prioritise tests, but not to exclude any of the three infections.

Enteric fever, bacterial sepsis, urinary tract infection and scrub typhus were important contributors in the negative group. Scrub typhus and other rickettsioses are now recognised as a major cause of AFI in India and merit routine inclusion in the diagnostic panel in the post-monsoon months, whereas the interpretation of the Widal test needs caution because of its limited specificity in endemic areas. The overall case fatality of 1.5% is low, and is consistent with the fact that most dengue and malaria deaths occur with delayed presentation, severe plasma leakage or cerebral and renal complications; the true malaria mortality in India is believed to be substantially higher than reported.

The clear monsoon-linked peak provides a window for pre-emptive vector

control, health-worker sensitisation and laboratory stocking of NS1/IgM kits, malaria RDTs and chikungunya IgM kits before June each year, in line with the global dengue strategy and national vector-borne disease control guidance[56]. Under-reporting of dengue remains a major concern hospital laboratories can narrow the gap by prompt notification of confirmed cases. Because a population-level serosurvey shows that infection is widespread the true community burden is certainly greater than the hospital burden documented here.

Strengths and limitations

The strengths of this study are the consecutive enrolment of a reasonably large sample, the use of multiple complementary tests for each infection, systematic evaluation of the negative group for other etiologies and the simultaneous assessment of three co-circulating infections. The limitations include its single-centre, hospital-based design, which may over-represent more severe illness; the lack of molecular confirmation and dengue serotyping; possible serological cross-reactivity among flaviviruses and persistence of IgM from earlier infection; the single-time-point sampling for most patients; and the absence of post-discharge follow-up, so that chronic chikungunya arthritis could not be assessed. Multi-centre studies with RT-PCR and genotyping are needed to confirm these findings.

CONCLUSION

Dengue, malaria and chikungunya together accounted for half of all patients presenting with acute febrile illness to this tertiary care hospital, with dengue the dominant pathogen, followed by malaria and chikungunya. Infections clustered during and after the monsoon, young adult males were most affected, and a small but clinically important proportion of patients had co-infections with a more severe course. Combining NS1 antigen with IgM serology for dengue, and peripheral smear with RDT for malaria, substantially improved detection, and the day of illness should guide the choice of test. Because the clinical features overlap, simultaneous laboratory testing for all three infections is advisable during the transmission season, and negative patients should be investigated for other treatable causes such as enteric fever, scrub typhus and bacterial sepsis.

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Conflict of interest and funding

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