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Adult Malaria and Co-Infections: Clinical Presentation, Diagnosis and Outcome in Central India

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ABSTRACT

Background: India has the highest malaria incidence in Asia and ranks second globally. Concurrent infections, particularly with dengue and chikungunya are common during monsoon and post monsoon seasons. These co-infections complicate diagnosis and management, especially in resource-limited settings. This study is aimed to investigate the profile, clinical features, and outcomes of co-infections in malaria patients.

Methods: A cross-sectional observational study was conducted in Central India, from June 2023 to October 2024. Patients presenting with acute febrile illness were tested by multiplex polymerase chain reaction for common causes of tropical fever. Clinical and demographic data were collected, and malaria cases with or without co-infections were included. Statistical analysis was performed with significance set at $p < 0.05$.

Results: Of 587 acute febrile illness patients tested via multiplex PCR, 214 diagnosed malaria cases included. Mono-infections accounted for 63.55%, while 34.45% had concurrent infections (51.28% males, 48.72% females). Co-infections were common with chikungunya (26.92%), dengue (24.36%), rickettsia (14.10%), and scrub typhus (11.54%). Fever lasted <7 days in 92.31% ($p < 0.001$) cases. Co-infected patients exhibited higher rates of symptoms like maculopapular rash, retro-orbital pain, and bleeding manifestations compared to mono-infected malaria cases. Co-infections showed severe thrombocytopenia, elevated liver markers, and complications like jaundice and abnormal bleeding. Mortality was observed in 07 (03.27%) cases.

Conclusion: This study provides valuable insights into the patterns, prevalence, and clinical implications of malaria co-infections in central India, emphasizing the critical role of advanced diagnostic techniques like multiplex PCR in improving early and accurate diagnosis. The findings underscore the complexity of febrile illnesses in high-burden regions and the necessity for tailored diagnostic and management strategies, particularly during monsoon and post-monsoon seasons. Future research with larger, multicentre studies and long-term follow-up is needed to validate these findings and address existing gaps in knowledge.

Introduction

Malaria remains a significant global public health challenge, though it has been successfully eliminated in 41 countries through proven case management and vector control strategies. Despite these achievements, the World Health Organization (WHO) reported a staggering 247 million malaria cases and 619,000 deaths worldwide in 2021. The WHO South-East Asia (SEA) region accounted for approximately 2% of the global malaria burden, with India alone contributing around 79% of malaria cases and 83% of malaria-related deaths within the region[1]. Notably, India accounts for 47% of the global *Plasmodium vivax* malaria burden, highlighting its strategic significance in global malaria elimination efforts, particularly within the SEA region[1]. The epidemiology of malaria in India is complex because of geographic and ecological diversity with multiple *Plasmodium* species and *Anopheles* vectors, and a wide variety of endemic settings[2]. Malaria in India is mainly caused by two major malaria parasites – *P. falciparum* and *P. vivax* (though cases of *P. ovale* and *P. malariae* have also been reported from some parts of the country). The disease is transmitted by nine *Anopheline* species, of which six are primary vectors[1].Malaria occurs year-round but reaches its peak during the monsoon and post-monsoon seasons. Occurrence of co-infections is very common and is attributed to a dense and diverse vector species and surge in their population during this period[3]. The most common co-infection is malaria with dengue, alongside bacterial and rickettsial infections. Leptospirosis, influenza A, Typhoid, rickettsiosis including scrub typhus, and chikungunya were some of the common tropical fevers reported in India[1]. These coinfections could arise due to chance because of host susceptibility and co-circulation of various disease agents. Malaria itself causes transient immunosuppression via impaired cell-mediated immunity, weakened phagocytic functions, reduced opsonisation, diminished cell-dependent cytotoxicity, and decreased humoral immunity, predisposing individuals to concurrent bacterial, viral and parasitic infections[4,5]. In endemic regions, concurrent infections frequently lead to diagnostic challenges. Shared clinical features, similar presentations, and overlapping laboratory results may be complicated by multiple infections within a single host, potentially confusing clinicians. This is particularly problematic in resource-limited settings, where confirming a suspicion of multiple infections may not be feasible[6]. These infections often go undetected and undiagnosed, causing delays in treatment and recovery, leading to morbidity and mortality[1]. Currently, immunosuppressed conditions like diabetes mellitus, hypertension, chronic kidney disease, malignancies and HIV infections are already a significant burden. A malaria infection could further exacerbate immunosuppression, increasing susceptibility to secondary infections[3]. Very few studies in Asia have focused on malaria and dengue co-infections, and not to mention research on other co-infections. Lack of quality clinical and laboratory data on co-infections among malaria patients despite several published systematic reviews of the literature was reported by Agrawal et al as well[7]. Therefore, it is difficult to effectively use data from existing systematic reviews or meta-analyses to make accurate diagnostic decisions when co-infections are present. Our study aimed to investigate the profile of co-infections in individuals with malaria to better understand this complex disease interaction. A discussion on the clinical features of these concurrent infections based on actual case reports from different countries in Asia might aid in creating increased awareness on the importance of these co-infections among communities, clinicians, and public health workers, as well as the regional health authorities. This will pave the way for relevant action plans to be initiated to address this health issue.

Methods

The present diagnostic, cross sectional observational study was carried out at MicroGenix Diagnostics, Amravati, India, from June 2023 to October 2024. Patients with undifferentiated acute febrile illness (AFI) were tested for common causes of fever by qualitative multiplex polymerase chain reaction (PCR). Demographic and clinical data were collected simultaneously at the time of receipt of sample. All the laboratory diagnosed malaria cases with or without evidence of concomitant infections were enrolled in the study. Approval from independent ethics committee was obtained with a waiver on patient's consent.

QUALITATIVE MULTIPLEX POLYMERASE CHAIN REACTION (PCR)

Whole blood (5 ml) from study subjects was collected in tube containing EDTA (Becton Dickinson vacutainer systems). Samples were transported to the laboratory at 2-8^0 C, and processed within 02 hours of collection. Nucleic acid extraction was performed on Mylab Compact XL, an automated extraction machine (Mylab Discovery Solutions, India) with Maverick Blood nucleic acid magnetic bead-based extraction kit (Mylab Discovery Solutions, India), using 500 microlitre of separated plasma. TRUPCR Tropical fever panel kit [(Multiplex Real-Time PCR Based detection, Version 2.0) (3B BlackBio Dx Ltd., India)] was used for qualitative detection and differentiation of Dengue virus, Chikungunya virus, ZIKA virus, West Nile fever (WNF) virus, *Salmonella* spp., *Plasmodium* spp., *Rickettsia* spp., *Orientia* tsutsugamushi, and *Leptospira* spp. Applied Biosystems QuantStudio 5 Real Time PCR (Thermo Fisher Scientific, US) was used. Assay was performed and interpretated as per manufacturer's instructions.

Peripheral blood smear examination, both thick and thin smears were examined in all the patients. Blood samples were collected from all patients and blood culture was done with automated blood culture system (BD BACTEC ™ FX 40, Becton Dickinson, USA). Bacterial identification and susceptibility testing was done with VITEK 2 Compact (bioMerieux, France).

STATISTICAL ANALYSIS

The data were collected, compiled, and analysed using EPI info (version 7.2). The qualitative variables were expressed in terms of percentages. Quantitative variables were both categorized and expressed in terms of percentages or in terms of mean, median and standard deviation. The difference between two proportions was analysed using chi-square or Fisher exact test. To test difference between two means, student t test was applied. All analysis was two tailed and the significance

level set at 0.05. ANOVA was applied in case of more than one group compared.

Results

From June 2023 to October 2024, a total of 587 acute febrile illness (AFI) patients were tested by multiplex PCR. Out of these, 214 diagnosed cases of malaria, were included in the study. Of 214 malaria cases, 136 (63.55%) had a mono-infection with malaria, while 78 (34.45%) had concurrent malarial infection. In the concurrent infection group, there were 40 males (51.28%) and 38 females (48.72%). The patients had a median age of 40 years, ranging from 18 to 80 years. Six patients (18.75%) had comorbid conditions: five with diabetes mellitus and one with bullous pemphigoid. The majority of patients were diagnosed with concurrent chikungunya (26.92 %) infection, which was significantly more common ($p < 0.05$), followed by dengue infection (24.36%) ($p < 0.05$), rickettsia spp. infection (14.10%) and scrub typhus (11.54%). Bacteraemia was observed in 10.26% cases, urinary tract infection (UTI) in 06.41% cases, lower respiratory tract infection (LRTI) in 03.85% and leptospirosis in 02.56% cases. [Table 1]. None of the patients were diagnosed with Zika virus infection and WNF and therefore are excluded from subsequent discussion.

Table 1: Distribution of Malaria and Co-infections

Total Number of Acute Un-differentiated Febrile Cases		587
Malaria Diagnosed Cases		214
Malaria Only		136
With Co-Infection		78
Plasmodium falciparum infection		54
Plasmodium vivax infection		82
Recovered		207
Death/Mortality		07
Co-infection with malaria (n=78)	**Frequency (n, %)**	**Associated organisms**
Dengue	19 (24.36)	Dengue virus
Chikungunya	21 (26.92)	Chikungunya virus
Zika Virus	00	Zika Virus
Leptospira ssp	02 (02.56)	*Leptospira ssp*
Rickettsia ssp	11 (14.10)	*Rickettsia ssp*
Scrub Typhus	09 (11.54)	*Orientia tsutsugamushi*
Bacteraemia	08 (10.26)	*Salmonella Typhi (03)*, *Escherichia coli* (03), *Staphylococcus aureus* (02)
Urinary Tract Infection	05 (06.41)	*Escherichia coli* (04), *Klebsiella pneumoniae* (01)
Lower Respiratory Tract Infection	03 (03.85)	*Klebsiella pneumoniae* (02), *Streptococcus pneumoniae* (01)

The average fever duration was 5.2 ± 1.79 days, ranging from 1 to 12 days, with a median of 4.5 days. Notably, 92.31% (n =72) of patients experienced fever for under a week, a statistically significant proportion ($p < 0.001$). Only 6 patients (07.69%) had fever lasting up to 10-12 days. Co-infections with bacteraemia, UTI and LRTI were suspected when symptoms were either persistent or newly emerging. New onset symptoms group included 01 case of leptospirosis, 02 cases of Rickettsia, 04 cases of scrub typhus, 03 cases of UTI and 02 cases of LRTI. Blood cultures revealed *Salmonella* Typhi (03), *Escherichia coli* (03) and *Staphylococcus aureus* (02), while other blood cultures were sterile. Organisms were isolated from sputum [*Klebsiella pneumoniae* (03), *Streptococcus pneumoniae* (01)] and urine [*Escherichia coli* (04), *Klebsiella pneumoniae* (01)] specimens in new onset symptom group patients.

Table 2 compares clinical features among patient groups. Symptoms observed in a mono-infection with malaria and concurrent malarial infection were fever (100%), myalgia (92.93%), headache (80.30%), and nausea/vomiting (42.93%). Those with malaria co-infections showed a higher incidence of maculopapular rash or eschar (54.84%) ($p < 0.0001$), retro-orbital pain (48.39%) ($p < 0.0001$), nausea/ vomiting (40.32%), arthralgia (35.49%), abdominal pain (17.74%), bleeding manifestation (12.90%), conjunctival suffusion (09.68%) ($p = 0.006$), and cough (04.84%) compared to malaria mono-infection group. Mortality was observed in malaria-dengue (10.52%), malaria-LRTI (33.33%) and malaria-chikungunya groups (4.76%), compared to the malaria mono-infection group, which had 3 deaths (2.21%). No fatalities were observed in other malaria co-infection groups.

Table 4 outlines complication rates among patient groups with malaria mono-infection and malaria co-infections. Anaemia (32.35%) and thrombocytopenia (45.59%) were prominent in malaria-only cases, while complications like jaundice (68.42%), and abnormal bleeding (47.37%) were more frequent in malaria-dengue co-infections. Severe thrombocytopenia was also significantly higher in dengue (36.84%) and chikungunya (38.10%) co-infections compared to malaria mono-infection. Acute kidney injury was most common in malaria-leptospirosis (50%) and malaria -UTI (40%) co-infections. Compared to malaria-only cases, malaria-dengue co-infections had higher rates of abnormal bleeding and jaundice, while bacteraemia co-infections showed increased circulatory shock (25%). Altered sensorium was present primarily in malaria-only cases (8.82%) with minor occurrences in dengue and bacteraemia co-infections. Severe complications like seizures occurred solely in malaria-only cases.

Table 2: Comparison of clinical features of the patients in Malaria mono-infection and co-infection group

Clinical Characteristics	Malaria Mono-Infection n (%)	Malaria with Co – Infection (n=78)							
		Dengue n (%)	Chikungunya n (%)	Leptospirosis n (%)	Rickettsia n (%)	Scrub typhus n (%)	Bacteraemia n (%)	UTI n (%)	LRTI n (%)
Fever	136 (100)	19 (100)	21 (100)	02 (100)	11 (100)	09 (100)	08 (100)	05 (100)	03 (100)
Fever duration (Days)	3-7	3-6	3-10	5-10	4-12	4-12	2-7	2-5	2-5
Headache	104 (76.47)	18 (94.74)	20 (95.24)	02 (100)	0	09 (100)	04 (50)	0	02 (66.66)
Retro-orbital Pain	0	15 (78.95)	13 (61.90)	02 (100)	0	0	0	0	0
Myalgia	131 (96.32)	16 (84.21)	21 (100)	02 (100)	02 (18.18)	09 (100)	02 (25)	01 (20)	0
Arthralgia	02 (01.47)	01 (05.26)	21 (100)	0	0	0	0	0	0
Nausea/Vomiting	60 (44.12)	11 (57.90)	12 (57.14)	02 (100)	0	0	02 (25)	00	0
Pain in Abdomen	13 (09.56)	04 (21.05)	03 (14.29)	02 (100)	0	0	01 (12.5)	01 (20)	0
Diarrhoea	17 (12.50)	01 (05.26)	00	00	0	0	02 (25)	0	0
Bleeding Manifestations	04 (02.94)	06 (31.58)	01 (04.76)	01 (50)	0	0	0	0	0
Rash	0	05 (26.32)	12 (57.14)	01 (50)	06 (54.54)	08 (100)	0	0	0
Eschar	0	0	0	0	02 (18.18)	0	0	0	0
Icterus	83 (61.03)	01 (05.26)	01 (04.76)	02 (100)	0	0	0	0	0
Conjunctiva suffusion	01 (00.74)	02 (10.53)	02 (09.52)	02 (100)	0	0	0	0	0
Convulsions	02 (01.47)	0	0	0	0	0	0	0	0
Altered Sensorium	04 (02.94)	0	0	0	01 (09.09)	0	0	0	0
Productive Cough	0	0	0	0	0	0	0	0	03 (100)
Pleural Effusion	0	0	0	0	0	0	0	0	01 (33.33)
Ascites	0	01 (05.26)	0	0	0	0	0	0	0
Splenomegaly	78 (57.35)	03 (15.79)	02 (09.52)	0	02 (18.18)	03 (33.33)	02 (25)	01 (20)	01 (33.33)
Hepatomegaly	79 (58.10)	12 (63.16)	08 (38.10)	02 (100)	03 (27.27)	02 (22.22)	04 (50)	02 (40)	02 (66.66)
Gasping Respiration	01 (00.74)	0	0	0	0	0	0	0	01 (33.33)
Mortality/Death	03 (02.21)	02 (10.52)	01 (04.76)	0	0	0	0	0	01 (33.33)

UTI: Urinary Tract Infection, LRTI: Lower Respiratory Tract Infection

Table 3: Comparison of laboratory features of the patients in Malaria mono-infection and co-infection group

Laboratory Features	Malaria Mono-Infection n (%)	Malaria with Co – Infection (n=78)							
		Dengue n (%)	Chikungunya n (%)	Leptospirosis n (%)	Rickettsia n (%)	Scrub Typhus n (%)	Bacteraemia n (%)	UTI n (%)	LRTI n (%)
Anaemia	44 (32.35)	04 (21.05)	05 (19.05)	0	01 (09.10)	0	01 (12.5)	0	01 (33.33)
Thrombocytopenia (cells/mm³)	62 (45.59)	12 (63.16)	11 (52.38)	01 (50)	08 (72.73)	04 (44.44)	0	0	01 (33.33)
Leukopenia	12 (08.82)	10 (52.63)	07 (33.33)	01 (50)	08 (72.73)	03 (33.33)	0	0	0
Neutrophilic leucocytosis	03 (02.21)	0	0	0	0	0	08 (100)	05 (100)	03 (100)
Mean haemoglobin	11.72 ± 2.12	11.35 ± 2.10	11.80 ± 2.15	11.23	11.47 ± 2.13	11.46 ± 2.21	11.67 ±1.97	11.92 ± 1.53	11.37 ± 1.6
Mean TLC	6409.70 ± 2464.57	6224.31 ± 2539.26	6329.53 ± 2403.21	8645.27	6793.28 ± 2934.89	5743.73 ± 2982.81	14890.41 ± 2227.52	12350.9 + 2892.53	11970.32 + 3584.76
Mean platelet count	103000.70 ± 63000	87981.79 ± 40467.58	98367.12 ± 49364.25	73000.62	89457.34 ± 0.453.89	96572.39 ± 54345.23	189349.12 ± 27845.67	176436.3 ± 10945.12	196436.3 ± 18674.6
Mean bilirubin	1.47 ± 1.25	1.28 ± 0.89	1.41 ± 1.18	1.68	1.43 ± 0.64	1.49 ± 1.26	1.24 ± 0.7	1.23 ± 0.5	1.29 ± 0.3
Mean SGOT	47.29 ± 18.32	88.45 ± 51.90	71.93 ± 40.83	58.36	69.49 ± 34.75	67.98 ± 29.58	37.88 ± 12.24	46.23 ± 11.29	39.91 ± 21.34
Mean SGPT	45.12 ± 32.74	73.32 ± 50.81	53.76 ± 39.78	59.41	78.23 ± 26.46	61.67 ± 31.45	47.98 ± 21.76	57.98 ± 19.73	51.84 ± 18.79
Mean creatinine (mg/dl)	1.42 ± 1.33	1.21 ± 0.29	1.39 ± 0.31	1.96	1.46 ± 1.2	1.41 ± 1.21	1.42 ± 0.7	1.34 ± 0.2	1.38 ± 0.6

UTI: Urinary Tract Infection, LRTI: Lower Respiratory Tract Infection

Table 4: Comparison of complications among patient in Malaria mono-infection and co-infection group

Complications	Malaria Mono-Infection n (%)	Malaria with Co – Infection (n=78)							
		Dengue n (%)	Chikungunya n (%)	Leptospirosis n (%)	Rickettsia n (%)	Scrub typhus n (%)	Bacteraemia n (%)	UTI n (%)	LRTI n (%)
Anaemia	44 (32.35)	04 (21.05)	05 (23.81)	0	01 (09.10)	0	01 (12.50)	0	01 (33.33)
Thrombocytopenia	62 (45.59)	12 (63.16)	15 (71.43)	01 (50)	08 (90.90)	04 (44.44)	0	0	01 (33.33)
Severe thrombocytopenia	28 (20.59)	07 (36.84)	08 (38.10)	0	02	01 (11.11)	0	0	0
Abnormal bleeding	08 (05.88)	09 (47.37)	02 (09.52)	0	0	0	0	0	0
Acute Kidney Injury	36 (26.47)	02 (10.53)	01 (04.76)	01 (50)	01 (09.10)	01 (11.11)	0	02 (40)	0
Jaundice	38 (27.94)	13 (68.42)	01 (04.76)	0	0	0	0	0	0
Hepatitis	57 (41.91)	08 (42.10)	01 (04.76)	0	0	0	0	0	0
Shock (SBP <90 mmHg)	10 (07.35)	02 (10.53)	0	0	0	0	02 (25)	0	0
Altered sensorium	12 (08.82)	02 (10.53)	0	0	0	0	01 (12.5)	0	0
Seizures	02 (01.47)	0	0	0	0	0	0	0	0

UTI: Urinary Tract Infection, LRTI: Lower Respiratory Tract Infection

Discussion

India has the second-highest malaria burden worldwide, following Africa. The monsoon and post-monsoon seasons are particularly favourable for malaria transmission and other vector-borne diseases. During these periods, individuals are likely to be exposed to various mosquitoes, including *Culex* spp, *Anopheles* spp, and *Aedes* spp, potentially leading to co-infections[3].In recent years many tropical countries have seen an unexpected rise and spread in cases of dengue and chikungunya[8]. The most recent systematic review had identified cases of malaria, dengue, and chikungunya co-infections; it also showed that malaria and dengue co-infection was the most common co-infection, followed by the dengue/chikungunya, malaria/chikungunya, and malaria/dengue/chikungunya co-infections[8]. In a present study, majority of malaria patients had concurrent chikungunya infection (26.92%). A systematic review and meta-analysis of malaria and chikungunya co-infection in febrile patients reported a varied prevalence of 0-10%[9]. In India, Morch K et al and Pandey S et al reported 8% and 3.7% prevalence of malaria and chikungunya co-infection respectively[3,10]. Higher prevalence of concurrent chikungunya infection in the present study could be attributed to ongoing chikungunya outbreak in addition to higher existing prevalence in our region. The most common infection during monsoon and post monsoon season in India is dengue fever, making it the most common concurrent infection as well[3]. A review of malaria-dengue coinfection cases in Asia by Selvaretnam et al, revealed that the majority of reports (26/36) were from India [11]. Previously published Indian data indicated varied rates from 1.54% to 10.25%, with an average around 4% of malaria-dengue co-infection [6,11-20]. In our study, 24.36% of malaria patients had concurrent dengue infections. These findings align with previously published data from India and other regions. In the present study, rickettsial spp and scrub typhus co-infections with malaria were found to be 14.10% and 11.54% respectively. In an epidemiological study in Africa, incidence of co-infection of *R. felis* and malaria was reported to be 6% in rural Mozambique, 11% in Nairobi, 23% in Senegal and as high as 79% in Kenya[21]. A systematic review and meta-analysis showed that the pooled prevalence of malaria and scrub typhus co-infection in febrile patients was 1%. Overall, the results showed that the pooled prevalence of scrub typhus infection among patients with malaria in India was 8% (nine studies with 59/794 cases)[22]. In India, the results of individual studies showed prevalence heterogeneity, the prevalence of 8.5% was reported in the study by Patil et al, [23] while the lower prevalence (1%) was found by Singh et al,[24]. Previously published meta-analysis showed that the pooled prevalence of malaria and leptospirosis co-infection among febrile patients was 1%. The pooled prevalence of leptospirosis infection among malaria patients was reported to be 12% in India[25]. Another recent study from India, reported 3.7% prevalence of malaria-leptospirosis co-infection in febrile patients[3]. In Andaman and Nicobar Islands, India, 6% prevalence of malaria-leptospirosis co-infection was reported[26]. In the present study, malaria-leptospirois co-infection were found in 02.56% cases. Data on invasive and secondary concomitant bacterial infections are well-documented in children, but comprehensive data for adults remain limited. In our study, 30.10 % of adults with malaria developed concurrent bacterial infections, with 10.25 % experiencing bacteraemia. Phu et al reported bacteraemia in 1.7% of patients with severe falciparum malaria[27]. Similarly, Das et al identified pneumonia, UTIs, and enteric fever as concurrent infections in severe malaria cases, accounting approximately 3.2% each [28]. Another study from India by Bhattacharya et al, found bacteraemia in 9% of adult malaria cases (all caused by *P. vivax*)[5]. A comprehensive multicentre study from India identified bacteraemia in 9.3% of malaria cases, with common pathogens including S. Typhi, S. Paratyphi, Gram-negative bacilli, and *Staphylococcus aureus*[10].

The clinical presentation of malaria typically includes fever with chills, malaise, anaemia, splenomegaly, thrombocytopenia, and organ involvement. These common clinical symptoms are also observed in dengue, chikungunya, scrub typhus, leptospirosis, and late-stage enteric fever. However, these illnesses exhibit certain distinguishing features, such as rash, conjunctival suffusion, lymphadenopathy, eschar, bleeding tendencies, and gastrointestinal or musculoskeletal involvement[3]. Studies by Barua et al and Mohapatra et al highlighted that in cases of co-infection, clinical features often align more with dengue. Anaemia, particularly haemolytic anaemia, in dengue cases suggests a concurrent malaria infection[18,20]. Moreover, dengue-malaria co-infections tend to present with greater severity in terms of clinical symptoms, morbidity, and laboratory findings, including anaemia, thrombocytopenia, jaundice, hepatitis, and renal dysfunction[11,20]. This increased severity is attributed to the heightened production of tumour necrosis factor-alpha and interleukin-6 in co-infections compared to single infections[20]. Our study revealed that concurrent malaria - dengue infection exhibited symptoms similar to classical dengue fever, with the addition of more severe thrombocytopenia during the febrile phase. It is notable that bleeding manifestations are uncommon in falciparum malaria, whereas in dengue haemorrhagic manifestations are common. However, when both the diseases can induce thrombocytopenia, it is difficult to decide which one is responsible for the causation of bleeding. Therefore, malaria with bleeding manifestations is considered as severe malaria and treated accordingly[18]. Previous studies revealed that chikungunya and malaria co-infection cases lacked distinguishable clinical features, with diagnosis relying solely on serology[3]. Similarly, a literature review highlighted the absence of clinical studies in Indian population. Concomitant malaria - chikungunya group showed chikungunya characteristic maculopapular rash (57.4%) and arthralgia (100%) in patients with malaria - chikungunya infection, however arthralgia was not as severe as it typically appears in chikungunya mono-infection. Reduced severity of arthralgia could be because of protection provided by *Plasmodium* species co-infection against chikungunya virus-induced pathologies[29]. Teo et al had demonstrated that concomitant infection with malaria and chikungunya limits the peak of joint inflammation with no effect on chikungunya viraemia. Reduced peak joint inflammation is driven by increased apoptosis of CD4+ T-cells in the lymph nodes and impaired CXCR3-mediated migration of these cells, preventing their infiltration into the joints. Delayed viral clearance from tissues is associated with a disruption of B cell affinity-maturation in the spleen that

reduces chikungunya virus neutralizing antibody production [29]. This explains the extended fever duration in malaria - chikungunya infection group in present study. Malaria - rickettsia and malaria - scrub typhus co-infection group presented with persistent fever and rash or eschar. A previous study showed that malaria and scrub typhus co-infection reduced hepatosplenomegaly or other organ dysfunctions, compared with malaria and scrub typhus mono-infections[22].Our findings are in accordance with this. However, only small groups of patients were investigated in previously published article and present study as well. The meta-analysis by Wilairatana P et al, revealed significantly reduced odds of co-infection, suggesting that malaria and scrub typhus co-infections occur randomly, rather than one infection increasing the risk of the other[22]. The observed association between malaria and scrub typhus may be attributed to the extensive geographic overlap of their distributions or the high prevalence of prior infections, which could lead to cross-reactivity and subclinical cases rather than a true co-infection prevalence. Additionally, scrub typhus infections might inhibit malaria or mitigate its clinical symptoms, though this possibility remains un-investigated and warrants further research[22]. Malaria-leptospirosis co-infections clinically presented like leptospirosis mono-infection, with nausea, vomiting, pain in abdomen, conjunctival suffusion, acute kidney injury, thrombocytopenia and leukopenia. Among patients with malaria and bacterial co-infection, there was a significantly higher incidence of anaemia, shock, acute kidney injury, and neutrophilic leucocytosis compared to those with malaria mono-infection. A study by Phu et al reported that bacteraemia was commonly associated with patients exhibiting a high parasite load (>20% parasitaemia), where the clinical presentation was dominated by severe malaria, manifesting as renal and hepatic dysfunction. They also noted that neutrophilic leucocytosis was an unreliable marker for bacteraemia, as it occurred in about 10% of cases and could also be observed in severe malaria[27]. Pandey S et al, identified neutrophilic leucocytosis in 4% of severe malaria cases and 14.3% of malaria-bacterial co-infection cases, including one case of leptospirosis. It was suggested that presence of a sepsis-like state in malaria, particularly in *Plasmodium vivax* infections, might indicate concurrent bacteraemia, especially in patients with underlying comorbidities[3]. In our findings, sepsis-like conditions with shock were observed in 50% of bacterial co-infection cases, aligning with previous reports.

Diagnostic tests available in India for AFI can be broadly divided into pathogen-specific and pathogen-nonspecific tests. Pathogen-specific tests are used to establish an etiological diagnosis of AFI. These tests can be further classified into various types, including antigen or antibody detection assays, molecular techniques for nucleic acid detection, and phenotypic methods for pathogen identification, such as smear microscopy for malaria or blood culture[30]. Peripheral smear microscopy remains the preferred method for malaria diagnosis, aligning with national guidelines that recognize it as the gold standard. Although its inexpensive, quantifies parasitaemia, identifies species; it requires skilled staff and asymptomatic parasitaemia in hyperendemic areas like India can confound diagnosis[31]. Rapid diagnostic tests (RDTs) are also acknowledged for quick detection. However, these RDTs show low sensitivities for low level parasitaemia (<100 parasites/µL) and cannot quantify parasitaemia. Varied performance is noted for different brands of RDTs. Furthermore, in areas where HRP-2 deletion *P. falciparum* exist, only pLDH based tests are effective[31]. RDTs are also available and routinely used for early diagnosis of dengue, scrub typhus, leptospirosis and enteric fever in India. Although, RDTs are inexpensive and can be used as point of care testing, they show reduced sensitivities in case of previous infections, varied performances across different brands, cross- reactivity and non-reactivity in secondary infections. Moreover, IgM RDTs cannot differentiate between acute and previous infection as IgM may remain elevated for 12 months post-infection. Serological tests like enzyme linked immunosorbent assay (ELIZA) are confirmatory, with 100% Specificity for ≥4-fold increased titre in convalescent sample, however, results are retrospective and of no use in management[3,30,31]. Culturing *Leptospira* is unreliable; therefore, the gold standard for diagnosis is serology confirmed by the microscopic agglutination test (MAT). MAT detects IgM and IgG antibodies against a pool of live antigens from various *Leptospira* serovars. A MAT titre > 100 is considered positive, but diagnosis is more robustly supported by either a fourfold rise in convalescent titres or a high single acute-phase titre (ranging from >200 to >1600, depending on endemicity). Following acute leptospirosis, both IgM ELISA and MAT remain positive for several years, with the duration varying between serogroups[10]. A prospective study in Barbados found positive MAT results up to 11 years after infection, with the highest prevalence in infections caused by the serogroup Autumnalis. In this group, 20% of cases had MAT titres exceeding 800 even four years post-infection[32]. For scrub typhus, ICMR guidelines suggest IgM ELISA for early diagnosis (sensitivity 66%, specificity 92%) or PCR/IFA for confirmation. Weil-Felix Test (WFT) shows inconsistent accuracy and is suboptimal for early detection due to cross-reactivity and late positivity. Despite limitations, WFT is widely used[30]. In typhoid diagnosis, the Widal test, commonly used in India, is unreliable due to variability and cross-reactivity. Isolation of *Salmonella* from the blood or bone marrow remains gold standard for diagnosis of enteric fever[31]. In the Indian context, the challenge with serology lies in the recurrent epidemics, which contribute to background positivity and cross-reactivity with other prevalent organisms such as scrub typhus and chikungunya. Consequently, serology should be utilized only in appropriate clinical settings. Although expensive, PCR should be prioritized for early and precise diagnosis of AFI as timely detection of bacterial diseases holds particular importance in guiding the targeted selection of antibiotics, mitigating inappropriate usage, and addressing antibiotic resistance concerns[3,8,33]. Routine screening for individual specific pathogens using single-target molecular tests in patients with AFI is both labour-intensive and time-consuming. This challenge can be overcome by adopting a molecular multiplex approach, which enables the simultaneous detection of multiple targets from a variety of pathogens. Multiplex PCR offers numerous benefits, including time efficiency, improved accuracy, cost-effectiveness, and high discriminatory sensitivity, making it a highly appealing choice for pathogen screening and monitoring[33].

Although, currently blood culture is considered to be a gold standard for diagnosis of blood stream infection (BSI), it takes one to several days to become positive and microbial identification and drug susceptibility testing require about 2 to 3 more days. Routine introduction of molecular testing to the diagnosis of BSI is also attracting attention as an effective means that can significantly change the treatment policy[34].A systematic review conducted by Timbrook et al, concluded that molecular diagnostic testing, which included identification of the organism and detection of resistance mechanisms, or both, was associated with reduced time to effective antibiotics, shorter length of stay, and decreased mortality rates when implemented alongside an antimicrobial stewardship program[35].

In the present study, mortality was observed in 7 out of 214 patients (03.27%). Among these, 3 deaths (2.21%) occurred in patients with malaria mono-infection. Among co-infections, dengue accounted for 2 deaths (10.52%), representing the highest percentage among the co-infected groups, followed by chikungunya with 1 death (4.76%). Notably, LRTI co-infection had the highest mortality rate within its small sample size, with 1 death (33.33%). Pandey S et al, reported mortality of 15.4% among malaria–dengue concurrent infection group and 2.4% in malaria mono-infection group[3].Favourable outcome, low mortality and less severe co-infections observed in the present study could be because of multiplex PCR, which helped in early and precise diagnosis by detecting low parasitaemia, viremia and bacteraemia, and therefore in timely accurate management of patients. However, confidence in the results is limited by the fact that reported numbers of deaths in the present study are insufficient to establish a significant difference. Furthermore, there is a possibility of non-inclusion of severely ill patients who were admitted to hospital, but succumbed to illness shortly afterwards before completion of laboratory diagnostic work up.

The study's strengths lie in its comprehensive investigation of malaria co-infections, covering a wide range of pathogens such as dengue, chikungunya, leptospirosis, scrub typhus, and bacterial infections by effectively utilizing multiplex PCR, enabling rapid and accurate detection of multiple pathogens. Multicentre data collection and year-round consecutive enrolment across all seasons enhance the extensibility of our findings in central India region. It provides insights into local prevalence rates and co-infection patterns, contributing valuable data to public health policies. Present study also meticulously correlates clinical presentations, diagnosis and outcome of malaria and co-infections. The study has several limitations. First, the sample size, particularly for some co-infection groups, is small, which restricts the applicability of the findings and limits the statistical power to establish significant differences. Additionally, the study lacks longitudinal follow-up, which could provide insights into the long-term outcomes of co-infections. The reliance on regional data limits its applicability to other areas with differing epidemiological profiles. Furthermore, while the study highlights the utility of multiplex PCR, it does not evaluate a detailed cost-effectiveness. These limitations suggest the need for larger, multicentre studies with robust designs to validate and expand on the findings.

Conclusion

This study provides valuable insights into the patterns, prevalence, and clinical implications of malaria co-infections in central India, emphasizing the critical role of advanced diagnostic techniques like multiplex PCR in improving early and accurate diagnosis. The findings underscore the complexity of febrile illnesses in high-burden regions and the necessity for tailored diagnostic and management strategies, particularly during monsoon and post-monsoon seasons. Despite its limitations, the study highlights the importance of a multidisciplinary approach in addressing co-infections, improving patient outcomes, and guiding public health interventions. Future research with larger, multicentre studies and long-term follow-up is needed to validate these findings and address existing gaps in knowledge.

Conflicts of interest

There are no conflicts of interest.

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