Original Article

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Microbiology Section

Seroprevalence and Co-infections of Different Infectious Aetiologies in Blood Culture Negative Febrile Patients Seeking Healthcare at a University Hospital in Northern India: A Cross-sectional Study

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ABSTRACT

Introduction: The burden of acute febrile illness remains underestimated in many low- and middle-income countries. The rationale of this study revolves around the gaps in diagnostics for acute febrile illnesses in these regions.

Aim: To highlight the seroprevalence and co-infections of different infectious aetiologies in blood culture negative febrile patients.

Materials and Methods: This cross-sectional study was conducted at Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India from October 2021 to July 2022, involving 153 serum samples from patients presenting with fever for less than two weeks duration during outpatient and emergency visits. The blood culture negative samples were further tested for other aetiologies such as Leptospirosis, Dengue NS1 antigen, Dengue IgM antibody, Chikungunya, Scrub typhus IgM, and Widal, respectively. Convalescent sera were also tested for all positive Widal results. All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) statistical software (IBM SPSS version 26.0, Armonk, N.Y.).

Results: Out of the 153 patients enrolled, 120 patients were tested for a panel of serological tests. The majority 52 (43.3%) were aged between 21 and 40 years. Fifty-five patients (45.8%) exhibited positivity, among which Scrub typhus was the most common aetiology with 21 (17.5%) case, followed by Leptospirosis 19 (15.8%), Dengue 10 (8.3%), Chikungunya 3 (2.5%), and Enteric fever 2 (1.7%), with no cases of malaria reported. Overall, co-infections reported in present study included 7 (5.8%) cases, of which scrub typhus and Leptospirosis were the most prevalent in 5 (4.16%) cases. Two patients (1.6%) presented with a co-infection of dengue and chikungunya.

Conclusion: The diverse aetiologies causing acute febrile illness necessitate a syndrome-based disease surveillance approach, accompanied by strong clinical acumen, for the betterment of patient outcomes.

Keywords: Febrile illness, Neglected tropical diseases, Serological tests

INTRODUCTION

India is home to a variety of aetiological agents causing Acute Undifferentiated Febrile Illness (AUFI) [1]. AUFI represents a diagnostic challenge in clinical practice due to its diverse aetiology and overlapping symptomatology. Characterised by the sudden onset of fever without an apparent source or specific clinical features, AUFI encompasses a spectrum of infectious and non infectious diseases, making accurate and timely diagnosis paramount for effective management and prevention of complications. In addition to bacteraemia, various neglected tropical diseases such as malaria, dengue, scrub typhus, chikungunya, and leptospirosis are important differential diagnoses [2].

The clinical presentation of AUFI often includes fever accompanied by non specific symptoms such as headache, myalgia, arthralgia, malaise, and sometimes gastrointestinal or respiratory symptoms [3]. This lack of specific clinical features makes it difficult to pinpoint the underlying cause based solely on clinical examination. Consequently, laboratory investigations and a systematic approach to differential diagnosis play a crucial role in identifying the causative agent. The non specific symptoms associated with these diseases, coupled with the lack of available diagnostic tools, further complicate the diagnostic process.

This study highlights a novel component of diagnosis by ruling out bacteraemia cases through blood culture and procalcitonin results. Additionally, various diagnostic modalities available have been discussed in detail. The study aims to estimate the burden of febrile illness in Northern India, highlighting common aetiologies and their changing epidemiologies over time. This research addresses the need to formulate a proper rationale for the diagnosis of febrile illnesses in our country, where the burden of infectious aetiologies is often underestimated. The aim of the study was to highlight the seroprevalence and co-infections of different infectious aetiologies in blood culture negative febrile patients.

MATERIALS AND METHODS

This cross-sectional study was conducted over a period of 10 months, from October 2021 to July 2022, at Sanjay Gandhi Post-Graduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India. The study protocol was approved by the Institutional Ethics Committee for Human Research at Sanjay Gandhi Post-Graduate Institute of Medical Sciences, with Registration Number 2021-109-IMP-EXP-38 and Reference Number PGI/BE/303/2021. Informed consent was obtained from the guardian for patients under 18 years of age.

Inclusion criteria: Blood samples of patients were primarily examined for blood cultures, which subsequently tested negative

after five days of incubation using the BACTEC blood culture system (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD®) were included in the study.

Exclusion criteria: The patients whose blood cultures were positive, patients with raised procalcitonin levels (>0.5 ng/mL) [4] and any lipemic and haemolysed samples were excluded from the study. Patients diagnosed with chronic and immunosuppressive illnesses such as tuberculosis, Human Immunodeficiency Virus (HIV), cancer, diabetes, or those undergoing chemotherapy were also excluded from the study.

Sample size: Out of 153 serum samples (after excluding lipaemic, haemolysed, and insufficient quantity samples), 120 samples from patients presenting with fever for less than two weeks in outpatient and emergency visits were considered.

Study Protocol

The serum samples from the included patients were tested using a panel of serological tests, including Malaria, Leptospirosis, Dengue NS1 antigen, Dengue IgM antibody, Chikungunya IgM antibody, Scrub typhus IgM, Widal, and Procalcitonin assay, to estimate the disease burden and co-infections [Table/Fig-1].

Tests	Kits used	Test performance
Malaria Rapid Ag test and ELISA*	Bioline, Human Malaria Antigen Elisa kit	Sensitivity: Pf- 99.7%, Pv- 95.5% Specificity: 99.5%
Dengue NS1 Ag ELISA and IgM	Panbio	Sensitivity: 76% Specificity: 98.4%
Leptospirosis IgM ELISA	Panbio	Sensitivity: 96.5% Specificity: 98.5%
Widal	Tulip widal	Sensitivity depends on local prevalence, specificity 100%
Scrub typhus	InBiOS	Sensitivity: 91.5% Specificity: 92.4%
Chikungunya IgM	J. Mitra	Sensitivity: 99.35% Specificity: 99.93%
Procalcitonin assay	Elesys BRAHMS PCT cobas	Limit of detection=0.02 ng/mL

[Table/Fig-1]: Test kits used in the study.
*Malaria Ag Elisa using serum samples though not recommended in routine testing, was done on 20 samples where whole blood samples were not received due to poor patient follow-up; Pf: *Plasmodium falciparum*; Pv: *Plasmodium vivax*

Procalcitonin, although a biomarker for bacterial infections, was tested in this study to rule out any other possibility of bacterial infection in blood culture negative samples. Since, it is a tertiary care hospital, by the time patients reach us, they have often already received antibiotics from local practitioners. Therefore, the chances of isolating bacteria from blood cultures further diminish. Procalcitonin levels were detected using a chemiluminescence assay [5]. Additionally, the serological tests requested by the concerned clinicians were negative when tested in the routine laboratory. Subsequently, these serum samples were assessed using a panel of tests beyond those initially conducted. The fatality rates were not compared.

Sample collection and transport: The blood samples received for any of the serological tests mentioned above were further processed using the automated blood culture system, specifically the BACTEC blood culture system [6] (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD). Blood was collected in a plain vial without anticoagulant, and serum was separated after centrifugation at 3000 rpm for five minutes. A minimum of 100 µL of serum was stored at -20ºC for present study. All blood cultures were sterile for the samples collected. Patients were followed-up, and blood in EDTA was collected for the malaria antigen detection test once the blood culture results were negative. There were 20 patients who did not follow-up and could not provide a blood sample for malaria testing; therefore, their serum samples were tested using a malaria antigen ELISA kit.

STATISTICAL ANALYSIS

Categorical variables were described using percentages, and all statistical analyses were performed using SPSS statistical software (IBM SPSS version 26.0, Armonk, NY).

RESULTS

Out of the total patients enrolled, 120 patients were tested for a panel of serological tests. The majority of patients 52 (43.3%) belonged to the 21-40 year age group. Females were affected more than males [Table/Fig-2].

	n (%)
Sample collected	153
Samples tested	120** (78.4)
Gender	
Male	58 (48.3)
Female	62 (51.7)
Age group (years)	
1-20	32 (26.7)
21-40	52 (43.3)
41-60	30 (25.0)
61-80	05 (4.2)
81-100	01 (0.8)

[Table/Fig-2]: Demographics of tested patients.
**14 samples were haemolysed; 11 samples were lipemic; Quantity of 8 was insufficient for processing

Overall, acute febrile illness accounted for 45.8% (55 out of 120) of cases identified by serological tests in all blood culture negative patients presenting with fever.

No cases of malarial aetiology were detected [Table/Fig-3].

Tests performed	Positive (%)
Malaria rapid antigen test	0
Dengue NS1 ELISA	0
Dengue IgM ELISA	10 (8.3)
ChikungunyaIgM ELISA	03 (2.5)
LeptoIgM ELISA	19 (15.8)
Scrub typhus IgM ELISA	21 (17.5)
Widal	2 (1.7)
	55 (45.8)

[Table/Fig-3]: Distribution of aetiological agents identified from patients seeking healthcare (n=120).
ELISA: Enzyme linked immunosorbent assay

In present study, 16 patients (13.3%) had raised procalcitonin values despite being blood culture negative. Seven patients with dengue presented with fever alone, while three experienced associated joint pain. Leptospirosis was present in seven patients with fever, in four with fever and chills, and non specific joint pain was observed in four patients. Enteric fever was detected in two patients, both presenting with fever and abdominal pain [Table/Fig-4].

Deranged liver enzymes (n=35, 29.16%) were an important finding while investigating the infectious aetiologies, followed by elevated urea and creatinine levels (n=15, 12.5%) in the majority of the population [Table/Fig-5].

Overall, co-infections reported in present study were 7 (5.8%), with scrub typhus and leptospirosis being the most common co-infection encountered 5 (4.16%). Additionally, 2 (1.6%) reported a co-infection of dengue and chikungunya.

DISCUSSION

Acute febrile illness opens the door to a wide spectrum of bacterial and viral illnesses that are neither tested nor discussed in great detail. The diseases such as malaria, dengue, enteric fever, scrub typhus,

Disease	Symptoms	Number of patients
Dengue	Fever	07
	Fever and joint pain	03
Leptospirosis	Fever	07
	Fever and chills	04
	Non specific joint pain	04
	Headache	02
	Abdominal pain	02
Scrub typhus	Fever	10
	Vomiting	02
	Malaise	01
	Diarrhoea	01
	Bleeding manifestations	07
Enteric fever	Fever with abdominal pain	02

[Table/Fig-4]: Clinical presentations of patients with reactive lab tests.

	Range of test results obtained	Mean±SD	Normal range
Haemoglobin (mg/dL)	2.1-16.2	10.36±2.55	12-16
Total leukocyte count (x1000µ/L)	3.7-31.8	11.25±5.63	4-11
Platelet count (x1000µ/L)	33-663	200.5±120.9	15-450
Alkaline phosphatase levels (IU/L)	31-1465	301.5±260.1	44-147
SGOT levels (U/L)	15-2217	150.6±292.2	8-45
SGPT levels (U/L)	11-1489	107.7±156.8	7-56
Amylase levels (U/L)	17-388	78.4±60.02	40-140
Conjugated bilirubin levels (mg/dL)	0.04-10.9	1.30±2.33	<0.3
Total bilirubin levels (mg/dL)	0.19-17.2	2.07±2.87	0.1-1.2
Albumin levels (g/dL)	1.5-7.1	3.95±1.12	3.5-5.5
Urea levels (mg/dL)	10-301	58.79±51.87	6-24
Creatinine levels (mg/dL)	0.3-15.1	1.81±2.41	0.7-1.3
Uric acid levels (mg/dL)	0.6-16.6	5.03±3.17	3.5-7.2
Sodium levels (mEq/L)	124-164	136.7±7.15	135-145
Potassium levels (mEq/L)	2.9-47	5.05±5.89	3.6-5.2
Protein level (g/dL)	3.1-9.4	6.20±1.34	6-8.3

[Table/Fig-5]: Laboratory parameters of the study population (n=120).
Median (SD), SGOT: Serum glutamic oxaloacetic transaminase; SGPT: Serum glutamic pyruvate transaminase; SD: Standard deviation

and leptospirosis are the primary considerations for diagnosis in patients presenting with acute febrile illness in a healthcare setting [7,8]. However, bacteraemia is always suspected as a priority when a patient presents with fever; if the culture report is negative, the patient is usually treated empirically to relieve symptoms [9]. The most affected age group in present study was 21-40 years. Similar findings were reported in the World Health Organisation (WHO) report on dengue in 2009 [10].

Overall, acute febrile illness accounted for 55 (45.8%) of all blood culture negative patients presenting with fever, as determined by serological tests. Scrub typhus was the most common aetiology 21 (17.5%) responsible for acute febrile illnesses, followed by leptospirosis 19 (15.8%), dengue 10 (8.3%), chikungunya 3 (2.5%), enteric fever 2 (1.7%), and malaria (no cases). Scrub typhus was identified as the most common cause of acute febrile illness in India, as reported in a study by Devasagayam E et al., [11]. Leptospirosis has emerged as one of the leading causes of febrile illness at our center, similar to findings by Sethi S et al., which highlight the increasing trends of leptospirosis in Northern India [12].

According to data published in the World Malaria Report 2021, India contributed 1.7% of global malaria cases and 1.2% of malaria-related deaths [13]. In India, malaria cases have consistently declined from 2.09 million in 2001 to 0.19 million in 2020 [14]. Similarly, no malaria cases were reported in present study. Furthermore, dengue NS1 testing revealed negative results for all patients under study, which can be attributed to the timing of the dengue NS1 testing after the blood culture results were negative, i.e., after 5-7 days. This ultimately led to poor antigen detection in serum after a 7-day period.

A study by Chrispal A et al., reported an incidence of 7% dengue fever in adult hospitalised patients in Southern India [15]. The results were comparable to present study, where the incidence of dengue fever was 8.3%. In present study, co-infections were reported in 7 (5.8%) patients, with the most common being scrub typhus and leptospirosis. A case of co-infection of scrub typhus and leptospirosis was reported in 2013 from Northeast India by Borkakoty B et al., [16]. The indirect Immunofluorescence Assay (IFA) is considered the gold standard in diagnosing scrub typhus [17]. However, the lack of availability of IFA at most diagnostic facilities underscores the need for improved early diagnosis of scrub typhus using ELISA and PCR.

In present study, 16 patients (13.3%) had elevated procalcitonin values despite being blood culture negative. This discrepancy can be attributed to variations in sampling techniques, timing of sample collection, antibiotic intake, or conditions that may lead to transient bacteraemia. Therefore, relying solely on blood culture and delayed non bacterial testing for acute febrile illness may prolong the patient's morbidity. Similarly, a study by Karlsson S et al., described the predictive efficacy of procalcitonin in sepsis [18]. This study highlights the importance of strong clinical acumen in diagnosing the overlapping symptomatology of acute febrile illnesses. Additionally, it is essential for clinicians to have knowledge of the appropriate diagnostic tests to perform within the correct timeframe for the prompt management of such patients.

Limitation(s)

This study had several limitations, including a small sample size, limited laboratory parameters, and the fact that it was conducted at a tertiary care centre where patients typically visit on referral from other healthcare settings. As a result, the data regarding their prior empirical treatment for illness is limited. Additionally, testing on convalescent serum samples could not be performed due to infrequent patient follow-ups.

CONCLUSION(S)

Scrub typhus, being the most common infectious aetiology in blood culture negative patients, is often underdiagnosed, which can lead to serious complications when it is presented late. The increasing incidence of leptospirosis cases serves as a critical alert for clinicians to consider this disease in their differential diagnoses, despite it being less frequently reported in our state. The study concludes by addressing an important concern: blood culture-negative febrile patients are usually underdiagnosed and often overtly treated with antibiotics. Therefore, the spectrum of different aetiologies should be clearly understood to effectively treat this population.

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