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Seroprevalence of Tropical Fever in patients with Acute Febrile Illness in a Tertiary care centre of North India

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ABSTRACT

Background and objectives: Tropical fever cause significant illness and debilitation, primarily in developing countries. Knowledge of their local prevalence can help in effective control and treatment programs. The aim of this study was to determine the prevalence of tropical fever in hospitalized patients and to correlate their clinical and serological profile.

Methods: A total of 6705 patients were enrolled in the study with history of acute febrile illness admitted in the hospital over a period of one year. Disease specific serological tests were performed to establish the diagnosis of tropical fevers. Serologically confirmed patients of tropical fever were studied for their clinical presentation and lab parameters.

Results: The Seroprevalence of Tropical fever was 26.3%. Dengue (17.3%) was most prevalent followed by scrub typhus and leptospirosis (2.6% and 2.2% respectively). Co-infections with dual etiologies were observed in 2.1% of cases. Tropical infections occurred most commonly during the monsoon and post monsoon months.

Conclusion: Tropical infections should be considered as important cause of acute febrile illness. Due to the overlapping clinical presentations, diagnosis must be confirmed by specific diagnostic tests. Possibility of co-infections must also be borne in mind when treating patients with acute febrile illness.

Key words: ELISA–Clinical profile–Seroprevalence–Seasonality–Tropical fever

1 INTRODUCTION

Fever is the commonest presentation of patients seeking healthcare in developing countries. In the developing world, the differential diagnosis for acute febrile illness (AFI) includes potentially significant illnesses such as malaria, dengue fever, enteric fever, leptospirosis, rickettsiosis, and other tropical infections. The Indian subcontinent by its very location represents one of the largest tropical and subtropical regions with many of these infections being prevalent.[1]

Based on literature search, infectious diseases which cause major burden of AFI in South East Asia include malaria, dengue, typhoid, chikungunya, leptospirosis and scrub typhus.[2–4] Differential diagnosis of these etiologies based on clinical criteria alone is not possible because of overlapping clinical presentations and the correct diagnosis is only possible by using pathogen specific diagnostic tests.

Confirmatory diagnostics play a key role in both patient management and evolution of epidemiology. However, most confirmatory diagnostics which are freely available in the tropics are based on serology. Their interpretation is further complicated by regional seroprevalence, delayed appearance of antibodies, variable but long term persistence of IgM antibodies, occurrence of cross reacting antibodies and early antibiotic treatment dampening the antibody responses. Therefore, definitive diagnosis requires demonstration of a four-fold rise in antibody titers against a causative agent within a specified period of time.[5] However, noncompliance of patients to report for a repeat serological testing after clinical improvement remains a major drawback in the serology based diagnostics. Although antigen based or PCR based diagnostics are increasingly introduced in order to overcome problems posed by serology-based diagnostics, their availability and affordability in the resource

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poor tropical countries is limited.[6]

Knowledge of local prevalence of infections is critical in guiding clinical work up and treatment. The exact burden of tropical infections in India is not fully known due to limited studies.[7] Therefore, while there is still a need to develop sensitive diagnostics for these infections, there also is an urgent need to create knowledge of the regional prevalence regarding tropical fevers, and intensify efforts to develop point-of-care diagnostics for tropical febrile illnesses in order to improve diagnosis and management of these diseases in developing countries. Keeping the above aims in mind, this study was undertaken to determine the prevalence of tropical fever.

2 MATERIALS AND METHODS

The study was conducted over a period of one year from April 2015 to March 2016 at a tertiary care teaching hospital in Punjab, India. During the study period, a total of 6,705 febrile patients of age >18 years admitted in the hospital with clinical suspicion of tropical fever (dengue, malaria, leptospirosis, scrub typhus, chikungunya and typhoid fever) were included in the study. Acute Febrile Illness(AFI) was defined as fever, documented as >38°C at the time of admission, on more than two occasions for at least 2 days but less than 14 days. A standardized clinical history and physical examination were performed on consenting patients by a trained clinician with the hospital admitting team.

Sample collection

Venous blood (2-5 ml) was collected into a sterile vacutainer containing anticoagulant potassium EDTA and a plain vacutainer without anticoagulant. Serum was separated by centrifugation of blood in plain vacutainer at 3000 g for 10 minutes as soon as possible and refrigerated (2-8°C) or stored frozen (≤ -20°C), if not tested within 2 days. Icteric, lipemic, or haemolysed samples were not used.

Tests Performed

Laboratory evaluations were selected to reflect a range of infectious diseases and were done as per the discretion of the treating physician. All the tests were performed and interpreted as per the manufacturer's instructions supplied in the kit.

Dengue: The Panbio Dengue IgM Capture ELISA (Australia Pty Ltd) and DENGUE NS1 antigen MICROLISA were used to detect IgM antibodies and dengue NS1 antigen in serum respectively.

Malaria: Blood samples were tested for malaria using the *SureTest* Malaria PF/PV HRP2/pLDH Combo rapid test. Thick and thin blood films stained with Giemsa were examined for blood parasites by oil immersion microscopy.

Leptospirosis: The Panbio *Leptospira* IgM ELISA (Australia, Pty Ltd.) was used for the qualitative detection of IgM antibodies to *Leptospira* in serum.

Scrub typhus: The Scrub Typhus Detect^{TM} IgM ELISA (InBios International, Inc.) was used for qualitative detection of IgM antibodies in human serum to *Orientia tsutsugamushi* (OT) derived recombinant antigen.

Chikungunya: The *Advantage* Chikungunya IgM card was used for rapid detection of chikungunya specific IgM antibodies in human serum.

Typhoid fever: Serological diagnosis of typhoid fever was done using Widal test. Agglutination titres ≥ 1:80 for O antigen and ≥ 1:160 for H antigen were considered as significant for evaluating the result of Widal test in the present study.

Culture of suitable body fluids (blood, urine, stool as the case may be), peripheral smear for malarial parasite, and acute and convalescent phase serology, in addition to basic investigations (CBC, Liver function tests, Renal function tests, etc.) were done.

Data collection and analyses

The data was entered in the designated proforma which included the socio-demographic characteristics (age, sex, etc.), presenting clinical symptoms, general physical examination, systemic examination and lab investigations performed. The data obtained was analyzed using descriptive statistics. The Chi-square test was used to find out the p values of the results. p value < 0.05 was considered significant.

3 RESULTS

During the study period, a total of 6,705 patients with AFI with clinical suspicion of tropical fever were admitted in the hospital. Out of these, 1,765 were serologically confirmed for the presence of tropical infection. The seroprevalence of tropical fever in this study was 26.3%. Dengue (17.3%) followed by scrub typhus and leptospirosis (2.6% and 2.2% respectively) were the more prevalent infections. (Figure 1) The prevalence of tropical fever was higher during monsoon and post monsoon season. (Figure 2)

Tropical infections were more common in the age group of 26-45 years. However, Malaria and typhoid fever affected the younger age group of 18-25 years more. Males (68.3%) were twice more affected by the tropical infections than females (31.7%).

Table I summarizes the clinical profile of patients diagnosed with tropical fever. Mean duration of fever was highest in patients suffering from typhoid (9 days) and least in chikungunya (3 days). Patients with tropical fever mostly presented with symptoms of myalgia (57.5%), vomiting (42.6%), abdominal pain (41.5%), arthralgia (23.7%), headache (18.6%) and jaundice (16.4%).

Lab investigations in patients with tropical fever revealed anemia predominantly seen in patients of malaria (45%). Leukopenia was seen mostly in patients of dengue (43%) whereas patients of leptospirosis, scrub typhus, malaria, typhoid, and chikungunya showed leucocytosis. Thrombocytopenia was predominantly seen in patients of dengue (93.1%) and malaria (82%). Transaminitis was common among patients of all tropical infections. Renal dysfunction with elevated serum creatinine was most common in patients of malaria (39.3%).

Shock was predominantly observed in patients of leptospirosis (8.8%) and scrub typhus (7%). Hepatic failure was commonly associated with leptospirosis (20.4%) and scrub typhus (21.5%). Renal failure was mostly seen in patients suffering from leptospirosis (11.5%). Encephalopathy was commonly seen in patients of malaria (6%).

Blood culture was done in 72 patients with positive Widal test, out of which 16 were culture positive. Of these 16 culture positives, 12 isolates of Salmonella Typhi and 4 isolates of Salmonella Paratyphi A were obtained. Out of 33 malaria cases, 63.6% had P. vivax infection and 36.4% had P. falciparum infection. Of these, 18 cases (54.5%) also had positive peripheral blood film.

In this study, 2.1% of patients had co-infections with more than one etiology. Most common co-infections were of scrub typhus and dengue (29%). (Table II)

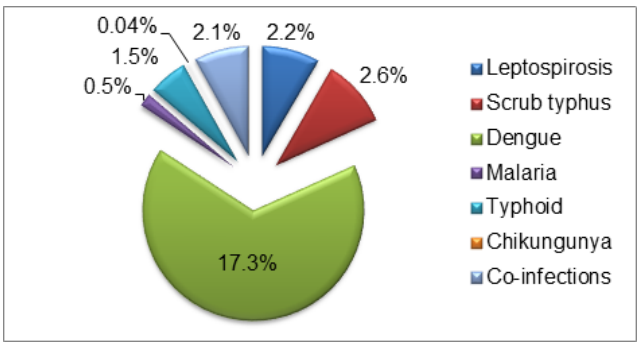

Figure 1. Distribution of various tropical infections in patients of AFI (n=1,765)

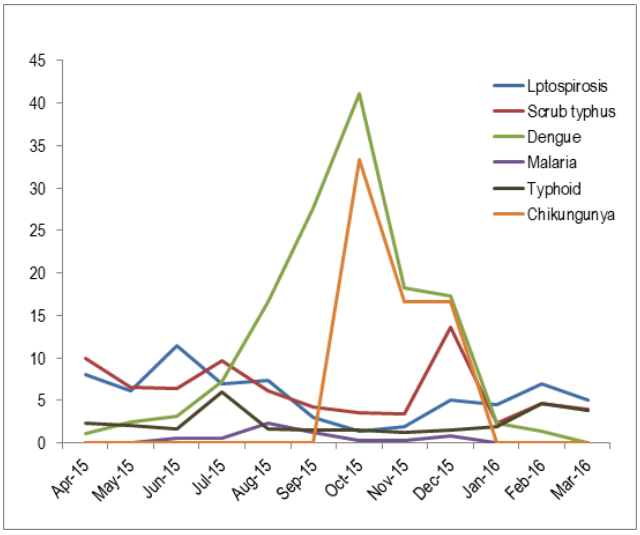

Figure 2. Monthwise positivity of Tropical fever

4 DISCUSSION

The seroprevalence of tropical fever in this study was 26.3%. Dengue (17.3%) followed by scrub typhus and leptospirosis (2.6% and 2.2% respectively) were the main infections. Less prevalent infections were malaria (0.5%) and chikungunya (0.04%). Other studies from Northern and Southern parts of the country by Chrispal et al, Gopalakrishnan et al and Kashinkunti et al have shown the similar results.[8–10] Difference in the prevalence rates may be attributed to different geographical areas with different climatic conditions.

Table 2. Spectrum of Co-infections (n=145)

	No. of patients	Percentage
Scrub Typhus + Dengue	42	29
Leptospirosis + Dengue	35	24.1
ScrubTyphus + Leptospirosis	25	17.2
Scrub Typhus + Malaria	11	7.7
Dengue + Typhoid	9	6.2
Scrub Typhus + Typhoid	8	5.6
Leptospirosis + Typhoid	8	5.6
Dengue + Malaria	5	3.4
Leptospirosis + Malaria	1	0.7
Dengue + Chikungunya	1	0.7
Total	145	100

In the present study, tropical infections were more common in the age group of 26-55 years. This age group comprises of the active working population who are most likely to be exposed to tropical infections. Also males (68.3%) were affected more than females (31.7%). This may be due to their easy exposure to mosquitoes and mites because of their outdoor activities. Similar findings have been reported in studies by Chrispal et al, Ittyachen et al and others, with most of the patients being in the productive phase of life (18–45 years) and a male preponderance.[8,10–11]

The seasonality of tropical infections during the monsoon and post monsoon months observed in our study is a well¯known documentation in other studies too. [8,12–14] It may be because this season is very favorable for high breeding of the vector mosquitoes. The spurt in the growth of scrubs in the post monsoon season creates a favorable environment for the mite populations resulting in high transmission rates of the infection. Heavy rainfall and water-logging also leads to contamination of drinking water.

The maximum seroprevalence was of dengue (17.3%) in our study. Studies from other parts of the country by Ghosh et al, Low et al, and Turbadkar et al have reported similar prevalence of dengue (17.9 %, 11.7% and 13.67% respectively).[15–17]Bleeding manifestations in the form of epistaxis, melena or hematemesis were observed in only 10.4% of patients in our study, which was statistically not significant. Khan et al also reported only 5% patients with bleeding while 40% patients had thrombocytopenia.[17] This is in contrast to study by Gupta et al which reported symptoms of melena (50%) and hematemesis (38%) during the

Table 1. Clinical profile of patients diagnosed with tropical fever (n=1,765)

Variable	Dengue (n=1165) (%)	Scrub typhus (n=172) (%)	Leptospirosis (n=147) (%)	Typhoid (n=100) (%)	Malaria (n=33) (%)	p value
Mean Fever (days) with SD	5 ± 2.38	7 ± 2.12	8 ± 2.12	9 ± 2.72	7 ± 2.23	<0.05
Myalgia	72.4	23.2	28.5	27	39.3	<0.05
Vomiting	50	11.6	25.1	15	36.3	0.09
Abdominal pain	38.6	29.6	40.8	3	66.6	<0.05
Arthralgia	34.4	1.7	3.4	0	0	<0.05
Headache	15.7	16.8	34	12	39.3	0.26
Jaundice	7.5	26.5	57.2	0	24.2	0.17
Bleeding manifestations	10.4	6.8	9.5	7	9	>0.05
Cough	5.3	35.4	5.4	2	9	<0.05
Decreased urine output	7	12.2	17.6	3	15.1	0.12
Abdominal distension	4.2	11.6	27.2	5	6	0.06
Breathlessness	4.1	26.7	7.4	2	12.1	<0.05
Diarrhoea	4.5	5.8	7.4	43	0	0.17
Altered sensorium	5	11	15.6	2	15.1	0.08
Hepatomegaly	27.4	25	53	48	27.2	0.12
Ascites	10.8	18.6	27.8	4	6.06	0.34
Rash	12.1	10.4	0.6	3	6.06	>0.05
Edema	6.6	11.0	20.4	4	15.1	0.16
Chest crepitations	2.6	38.9	6.1	2	6	0.24
Splenomegaly	1.2	5.2	6.1	13	57.5	0.07
Anaemia (Hb <10mg/dL)	22	9.8	18.3	9	45	0.24
Leukopenia (TLC<4000 cells/μL)	43	7	2.7	5	18	0.11
Leukopenia (TLC >11000 cells/μL)	7	59.3	68.7	61	30	0.36
Thrombocytopenia(<100×103 cells/μL)	93.1	64	52.3	47	82	0.13
Hepatic failure	3.7	21.5	20.4	7	3	0.35
Shock	4.8	7	8.8	6	3	0.11
Encephalopathy	4.8	3	5.4	2	6	0.74
Renal failure	1.8	7	11.5	1	9	<0.05
ARDS	0.4	1.1	2.7	0	3	0.01
Sepsis	0.1	1.7	1.3	2	0	0.04

2006 outbreak of dengue in North India.[18]

The prevalence of leptospirosis in this study was 2.2%. Prevalence rates reported by other studies from various parts of the country are highly variable with a higher prevalence rate from south India (1.3% by Bawane et al, 11.4% by Sahira et al, 12% by Prabhakar et al).[19–21]

Common clinical symptoms in patients of leptospirosis in our study were jaundice (57.1%), abdominal pain (40.1%), abdominal distension (27.2%) and myalgia (28.5%). Neurological manifestations in the form of altered sensorium were seen in 15.6% of leptospirosis patients. Such manifestations are varied and often lead to misdiagnosis, unless strongly suspected.

The prevalence of scrub typhus in our study was 2.6%. Other studies conducted in different parts of India have reported prevalence rates ranging from 30.8% to 46%.[22,23] Low prevalence in our study may be due to the fact that ours being a dry area with scanty rainfall and vegetation is not supportive of scrub typhus as it occurs mostly in rainy and hilly areas with moisture and scrub vegetation.

Erythematous rash was present in 10.4% cases of scrub typhus in this study, however no case with eschar was observed. Although, presence of eschar is indicative of scrub typhus, its absence does not rule out the diagnosis. Also, it is difficult to visualize it on dark skinned individuals. Eschar has been found to be uncommon in most studies from South East Asia and Indian subcontinent which was in concordance with our findings.[24,25]

In our study, 63.6% cases had *P. vivax* infection and 36.4% had *P. falciparum* infection. Our findings are supported by Singh et al [26] who reported *Plasmodium vivax* (54.76%), *Plasmodium falciparum* (17.80%), and mixed species (27.44%) but different from Basavaraj et.al who reported predominance of *P. falciparum* infection as compared to *P. vivax* (55.3% and 44.7% respectively).[27] The difference in prevalence of Plasmodium species in different areas can be due to varying endemicity of a particular type and higher relapses in vivax type.

The prevalence of typhoid in our study was 1.5% on the basis of significant Widal titers. The lab parameters of most cases of typhoid showed leukocytosis (61%). This is in contrast to other studies which have reported leukopenia in most patients suffering from typhoid.[28,29] This may be explained by the fact that bacteremia in typhoid results in

elevated white cell count as 16 patients of typhoid had positive blood culture for *Salmonella* species in our study. Also intake of antibiotics prior to admission may result in leukocytosis.

Regarding clinical symptoms, signs, hematological alterations, and complications, it was difficult to differentiate among leptospirosis, dengue, scrub typhus and malaria due to the overlap in clinical symptoms, signs and lab parameters. (Table 1) There was significant difference of only a few clinical symptoms among tropical fever. For example, myalgia and arthralgia were common presenting symptoms in cases of dengue (72.4% and 34.4% respectively). Similarly, cough and breathlessness (35.4% and 26.1% respectively) were common in patients of scrub typhus. Some pathologies were represented by few cases (e.g., only three chikungunya cases) making it difficult to establish a definitive conclusion for this disease. Furthermore, the sensitivity of tests used may have been low or we may have sampled at the wrong time. We could not find any similar study comparing the clinical profile of various tropical infections in patients of AFI.

In this study, 2.1% of patients had co-infections with more than one etiology. Most common co-infections were of scrub typhus and dengue (29%) followed by leptospirosis and dengue (24.1%), scrub typhus and leptospirosis (17.2%). Similar co-infections have been reported in other studies. Ahmad et al reported 49 out of 200 patients (16.4 %) with evidence of infection by more than one organism. Scrub typhus, dengue and malaria were seen in various combinations.[30] Mittal et al also reported mixed infection in 1.88% of febrile patients; most common mixed infections being dengue with scrub typhus (31%), malaria with scrub typhus (23%) and malaria with dengue (16.6%).[31]

Presence of such co-infections can be explained by the fact that all the infections studied are arthropod borne diseases with a common mode of transmission. During monsoons, heavy rainfall, water-logging and growth of vegetation promote the proliferation of the vectors in general. The predisposed individuals are exposed to the various vectors, leading to transmission of multiple organisms. Cross-transmission by a single vector due to hybridization and/or mutation due to pesticide use may also have contributed.

Co-infections whether true or due to serological cross-reactivity could not be ascertained due to non-availability of further confirmatory tests like polymerase chain reaction (PCR), MAT, IFA and/or culture for confirming the presence of an organism.

Definitive diagnosis by serology requires demonstration of a four-fold rise in antibody titres against a causative agent within a specified period of time. However, non-compliance of patients to report for a repeat serological testing after clinical improvement or loss to follow up following discharge or death of the patient remains a major drawback.

In the resource-constrained tropical settings, specific serological tests on a single serum sample may aid in early diagnosis of tropical infections. Hence, despite the above limitations their role in timely management of patients cannot be overlooked.

5 CONCLUSIONS

Tropical infections should always be taken in consideration while diagnosing a patient of acute febrile illness. Differential diagnosis of these aetiologies should not be based on clinical criteria alone because of significant overlap in their clinical presentations. Specific lab diagnostic tests guided by detailed clinical history and examination play a vital role in establishing the diagnosis of tropical fever.

In addition, knowledge of local prevalence and recent outbreaks in different geographical areas can be very helpful in recognizing the clinical entity. Possibility of co-infections with one or more causative organisms must also be borne in mind when treating patients with acute febrile illness.

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