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Interpretive challenges in serodiagnosis of leptospirosis and dengue in South India

[1] Swapna Kurup Rajgopal, [2] Kousalya M

[1] Associate Professor, Dept. of Microbiology, Karpagam faculty of medical sciences and research, Coimbatore, Tamil Nadu, India
[2] Tutor, Department of Microbiology, Karpagam faculty of medical sciences and research, Coimbatore, Tamil Nadu, India

Abstract
Objectives: This study was undertaken to correlate the clinical and laboratory findings for both the diseases and gain some understanding in the challenges faced in the interpretation of these findings.
Methods: 296 patients with history of fever were tested for dengue and leptospirosis simultaneously using Pan-bio Dengue IgM capture ELISA and Pan-Bio Leptospira IgM ELISA. Based on the test results, the patients were divided into 4 groups and all records studied for comparable clinical and laboratory data.
Results: 6.7% of the patients were found to seropositive for both dengue and leptospirosis. Fever, myalgia, headache, jaundice, nausea and vomiting were the commonest presentations accompanied in lower frequency by thrombocytopenia, hepatomegaly and raised transaminases. Hemoconcentration (44.8%) and thrombocytopenia (55.5%) were observed with dengue fever. 10.8% of patients with leptospirosis presented with thrombocytopenia.
Other co-infections and underlying conditions included Hepatitis A E and B, enteric fever and complications as Multiorgan failure, Pneumonia and Altered sensorium.
Conclusions: Many cofactors such as acute renal failure altered sensorium and severe hemorrhagic episodes were found to be the cause of increasing morbidity and similar clinical presentations which often confounded the judgment for early management. More analysis is required to find the cause of cross-reactivity between the ELISA kits for different infections for use in endemic regions.

Keywords: ELISA, Co-infection, Laboratory parameters, Interpretation

1. Introduction
Many studies on seasonal infections have been done in India with respect to water borne and vector borne diseases. Initial presentations can be similar making it difficult for management unless few laboratory results confirm the diagnosis causing a further delay in treatment or in few cases complications warranting admission in ICU.
Dengue has been a major cause of morbidity and mortality in the tropical and subtropical regions of the world along with Leptospirosis, fulminant HAV hepatitis, typhoid and malaria. In populations living in areas of endemicity, most dengue virus infections are difficult to diagnose as they are asymptomatic [1]. Classic Dengue fever (DF), an acute presentation of fever, headache, prostration, leukopenia and thrombocytopenia, accompanied with symptoms such as myalgia, arthralgia and a macular skin rash [1]. The major pathophysiologic hallmark that distinguishes Dengue hemorrhagic fever (DHF) from DF is plasma leakage due to an increase in vascular permeability. Of late there have been reports of fulminant hepatitis with high mortality in-patients with dengue infection [2].
Leptospirosis has a higher incidence due to flooding during the monsoon season and natural disasters [3, 4]. The positivity rate ranged from 3.27% in the central zone to 28.16% in the southern zone in India [5]. Patients present with sudden onset of fever, chills, and headache. The frontal throbbing headache is accompanied by retro-orbital pain and photophobia. A common presentation conjunctival suffusion is uncommon in other infectious diseases. Thrombocytopenia though not directly correlated with haemorrhage, has been correlated with the development of acute renal failure [6, 7, 8]. Oliguria is a significant predictor of mortality [9].
Many authors have documented outbreaks of Leptospirosis such as from along the coastline of west and southern India and the Andaman Islands [10, 11]. Indian prevalence rates are difficult to estimate as concurrent infections maybe present with similar clinical presentations, therefore challenging from a clinical perspective. The study was undertaken to evaluate the challenges faced by laboratory diagnosis of these two diseases correlating retrospectively with biochemical and serological parameters and to emphasize the underestimating of the incidence of the diseases.

2. Materials and methods
A retrospective case analysis of the study was conducted in Kochi over a period from July 2009 to November 2010 covering two monsoon seasons in a tertiary care hospital specializing in gastroenterology and oncology with expanding clientele in the other departments.
All patients whose samples were sent for diagnosis for both Leptospirosis and Dengue were included in the study and all records studied for comparable clinical and laboratory data.
The diagnosis of DHF in accordance with World Health Organization criteria was adapted with fever>38ºC, low platelet count (<100,000 /uL), hemorrhagic tendency and evidence of capillary leakage (any hematocrit increase of 20% from baseline, pleural effusion, ascites) [1].
Sera were processed using anti- Leptospira IgM ELISA and The Dengue IgM capture ELISA (Pan Bio, Brisbane, Australia) as screening tests. Tests were performed according to manufacturer's instructions employing 10 µL of test serum. Readings interpreted as per instructions and recorded as positive if the value > 11, intermediate between 9-11 and negative <9. In cases seropositive for both dengue and

leptospira tests, tests were repeated after 3-7 days to confirm diagnosis.

The study groups were divided into
1. Leptospirosis positive (83 patients)
2. Dengue positive (49 patients)
3. Both Dengue and Leptospirosis seropositive (20 patients)
4. Both dengue and Leptospirosis seronegative (144 patients).

In each group, co-infections with Hepatitis A, B, C and E, HIV and Salmonella sp (Widal test) were noted. Any other underlying cause for requiring admission or OPD visit was also noted. Hepatic involvement was evaluated as 1. Normal levels of aminotransferase; 2. Elevated levels of one/both enzymes. Direct bilirubin test, Creatine kinase levels, Gama-glutamyl transpeptidase, Lactate dehydrogenase or Alkaline phosphatase. Reports of peripheral blood smear results checked and outcome of the treatment was noted.

3. Statistical analysis

All the data was entered and analyzed in Microsoft Excel 2010.

4. Results

Specimens from 296 patients tested between July 1 2009 and November, 2010, were evaluated for Leptospirosis and Dengue infection and retrospectively clinical diagnosis correlated with the laboratory findings. Patients who were tested for only one of the diseases (Dengue or Leptospirosis) were excluded from the study.

Patients seropositive for Leptospirosis reported a longer duration (3-8 days) of fever than patients with Dengue. While most patients with Dengue or Leptospirosis had continuous fever, intermittent fever was also noted in patients with Leptospirosis. Aside from fever and rash, few patients with leptospirosis and dengue had similar symptomatology (Table 1) as headache, myalgia, nausea, and vomiting common in both.

Table 1: Symptomatology within various groups (percentage in brackets)

Manifestations	Leptospirosis N=83	Dengue N=49	Seropositive both N=20	Seronegative both N=144
Male	46 (55.4)	26 (53)	14 (70)	94 (65.2)
Fever	80 (96.3)	49 (100)	16 (80)	121(84).
oliguria	25 (30.1)	1 (2)	7 (35)	6 (4.1)
Headache	49 (59)	42 (85.7)	10 (50)	34 (23.6)
Myalgia/bodyache	44 (53)	34 (69.3)	12 (60)	70 (48.6)
Conjunctival oedema	46 (55.4)*	-	1 (5)	-
Rash	12 (14.4)	29 (59.1)	4 (20)	2 (1.3)
Abdominal pain/ diarrhoea	8 (9.6)	11 (22.4)	5 (25)	19 (13.1)
Nausea/ Vomiting	21 (23.3)	13 (26.5)	5 (25)	33 (22.9)
Hepatomegaly	16 (19.2)	9 (18.3)	6 (30)	24 (16.6)
Jaundice	33 (39.7)	12 (24.4)	7 (35)	24 (16.6)
Mortality	11 (13.2)	5 (10.2)	1 (5)	4 (2.6)
Tourniquet test	10/41(24.3)	35/49 (71.4)*	11 (55)	-
Widal test positive	3 (3.6)	-	3 (15)	10 (6.9)
AG Reversal	7 (8.4)	7 (14.2)	5 (20)	11 (7.6)
Raised transaminases	12 (14.4)	20 (40.8)	3 (15)	73 ALT (50.6)
Thrombocytopenia	9 (10.8)	27 (55.1)	9 (45)	7 (4.8)
Renal failure	10 (12.0)	2 (4.4)	1 (5)	7 (4.8)

Leptospirosis was positive in 28% of the patient samples, dengue in 16.55% and among the patients seropositive for both infections, dengue was clinically suspected in 11/20 patients. Out of these 20 patients, 3 of clinically suspected dengue cases had the seropositive values for leptospirosis in the intermediate range [9, 11].

Our study was undertaken to correlate the clinical and laboratory findings of patients admitted with pyrexia of unknown origin, flu-like illness and other symptoms highly suggestive of either leptospirosis or dengue infection. Monocytosis was more noted in group 2 (30.6%) while neutrophilia was noted (56.6%) in group 1. Hemoconcentration (44.8%) and thrombocytopenia (55.5%) were observed with dengue fever. 10.8% of group 1 presented with thrombocytopenia.

40 of group 2 had classical dengue fever (DF) while 8 were in grade 1/2 DHF and 1 of grade 3 Dengue Shock Syndrome, according to the traditional WHO criteria [1]. Based on various criteria, the group 2 were classified according to revised WHO classification. (Table 2) [18].

Table 2: According to the revised classification of the Dengue guidelines, (24)

Classification	Symptoms and signs	Total
Dengue with fever without warning signs	Nausea vomiting	13
	Rash	34
	Bodyache, myalgia	23
	Leukopenia	16
	Positive tourniquet	35
Dengue with Warning Signs : Dengue as defined above with any of the following	Abdominal pain or tenderness	6
	Persistent vomiting	1
	Clinical fluid accumulation	2
	Mucosal bleeding	16

	Lethargy, restlessness	10
	Liver enlargement. 2 cm	2
	Laboratory: increase in HCT concurrent with rapid decrease in platelet count	
Severe Dengue with at least one of the following criteria:	Severe Plasma Leakage leading to: – Shock (DSS)	1
	Fluid accumulation with respiratory distress	1
	Severe Bleeding as evaluated by clinician	3
	Severe organ involvement	
	Liver AST/ALT>1000	5
	CNS impaired consciousness	7
	Failure of heart or other organs	3

The patients with underlying conditions in group 3 consisted of genital TB (1) Diabetes (3), ethanol related hepatitis (2), bronchopneumonia (3) and Crohn's disease (1). Peripheral blood smear in 18.1% of group 3 recorded neutrophilia, mild-severe thrombocytopenic picture with dimorphic anemia and polychromasia and (9%) erythroblasts with plasmacytosis.

Of the 62 patients presenting with fever and acute hepatitis, two clinically suspected leptospirosis could not be laboratory confirmed with the ELISA. They developed multi-organ failure, with pancytopenia/ thrombocytopenia and in one patient complicated with rhabdomyolysis, febrile convulsions and metabolic acidosis. (Table 3)

Table 3: Patients with fever and hepatitis

Co-factors	Leptospirosis N=14(16.8%)	Dengue N= 7 (14.2%)	Seropositive both T=4 (20%)	Seronegative both=37 (25.6%)	Total n=62
HAV	5	3	2	19	29
HEV	2	1		5	8
HBV	4			3	7
HCV				1	1
Alcohol			1	7	8
Drug				4	4
Other Infection	3	2	2	1	8

The demographic data showed the variation of age ranged from 16 to 80 years (male) and 17 to 75 years (female) with the male to female ratio to be 1.78:1. The age and sex distributions were comparable between the four study groups. 7.09% of patients had fatal outcome due to many factors. (Table 4).

Table 4: Demography of the groups with fatality

Variable	Leptospirosis N=11(13.2%)	Dengue N= 5(10.2%)	Seropositive both =1(5%)	Seroneg both=4(2.7%)
Underlying disease/intervention				
HAV	2 (18.1)			
HBV	1 (9)			
HEV			1 (100)	
On Mechanical ventilator	8 (72.7)	5 (100)	1 (100)	4 (100)
Heart disease	1 (9)	1 (20)		
DM	1 (9)		1 (100)	1 (25)
HT	1 (9)			
Cirrhosis				
Complications				
Septicaemia	3 (27.2)	1 (20)		1 (25)
Acute renal failure	9 (81.8)	1 (20)		3 (75)
Acute hepatic failure	8 (72.7)			3 (75)
Hemorrhagic		4 (80)	1 (100)	
Pneumonia	4 (36.3)	1 (20)	1 (100)	
Altered sensorium	5 (45.5)	4 (80)	1 (100)	3 (75)

5. Discussion

In a developing country like India, where waterborne and airborne diseases are endemic, zoonotic diseases are becoming more prevalent during monsoon season. To reduce the turn-around time of diagnosis between the time of admission and confirmation of the clinical diagnosis, a wide array of tests are prescribed for the patients, often in patients with pyrexia of unknown origin, critical care parameters or in the vulnerable age group. Classical definitions guide the clinicians to a certain diagnosis but most clinicians broaden their management to include one or two more diseases until the laboratory confirm their diagnosis. The investigative kits usually supportive of the clinical diagnosis may in some cases can reveal more confusing results. With delay or misdiagnosis, the risk of developing complications increases hence the need for intensive care pertinent in many cases.

Dengue infection may present as dengue fever or more severe dengue hemorrhagic fever dengue shock syndrome. Clinical presentations of Leptospirosis may vary from flu-like illness to severe hepatic and renal involvement. Patients rarely present to the OPD or emergency department early in the disease. It is common to investigate simultaneously for number of causes presenting with similar symptomatology such as Widal test for enteric fever, Weil Felix test for

Rickettsia and peripheral smear for malarial parasite. Local prevalence usually will guide the diagnosis and initiate the preliminary management of the disease. The sensitivity and the specificity of the laboratory investigations is vital to correct and timely diagnosis and preventing a panacea-like management to prevent complications if any.

The laboratory at Mahidol University tested the Pan-bio ELISA Dengue kits against 12 serum samples from patients with leptospirosis. The Pan-bio ELISA showed cross-reactivity with 58% of these samples. [12] Compared to this study, the cross reactivity in our study was not high. Studies have reported co-infection between Dengue and other flaviviruses and other acute febrile illnesses including leptospirosis [12, 13].

Dengue virus specific IgM antibodies tend to appear around 3 days after infection peak in 2-3 weeks and wanes over next few weeks [14, 15]. As IgM is known to persist for more than 60 days, IgM assays should not be used in dengue-endemic countries as confirmatory tests for current illness. Increased specificity in these tests is needed to remove the cause of the cross-reactivity. IgM tests can be useful for screening of acute infections.

There was a male preponderance in each clinical group which was comparable with other studies. Surveillance data from Malaysia revealed a male preponderance among Indian and Malay patients (1.5:1), but the ratio was almost equal for those of Chinese origin [16]. Common presenting features in all the groups were fever headache, bodyache, rash, myalgia nausea and vomiting. Table 1 gives the relative representation of the symptoms in each group comparable with prevalence in other studies [17].

While using the new revised classification for dengue increased and clarified the parameters to classify into 3 groups [18], using the traditional system helped to focus on a particular presentation based on the risk factors. (Table 2)

The results of the present study (Table 3) show that many patients with dengue viral infection had some degree of liver involvement as indicated by the abnormal liver function test results, especially in the serum transaminase levels. The liver is often involved in dengue viral infection, thus dengue fever should be suspected in cases of fever with acute hepatitis where the rates of infection are high [2]. The prevalence of hepatic involvement in both the disease was similar.

In leptospirosis liver is often affected, the evidence supported by histopathological and immunohistochemical studies. Hepatocyte apoptysis, hepatocellular damage and disruption of hepatocyte intercellular junctions may account for the elevated indirect bilirubin levels in icteric forms of leptospirosis [4,6]. In our study, co-infection with Hepatitis A, B and C viruses or history of drug induced and alcohol associated liver injury were noted in patients who presented with fever and hepatitis. The elevated enzymes or bilirubin levels could be consistent with these co-factors also so definitive association with Dengue or Leptospirosis could not be made.

We noted the association of Multiorgan failure with mortality in leptospirosis, often in patients admitted with altered sensorium or complicated by septicaemia which was found in other studies also (Table 4). Acute renal failure, oliguria, respiratory insufficiency and leukocytosis are known to increase the risk of a fatal outcome. [6, 7, 8, 9].

Dengue fever is associated with thrombocytopenia even in mild and uncomplicated cases [1, 2]. Hemorrhagic episodes accompanied with altered sensorium and mechanical ventilator assistance increased the chance of death in patients with Dengue as also reported in similar studies. [2] Our study data regarding the clinical presentation s in Dengue were found comparable with other similar studies. Hemorrhagic presentations were not seen in the Leptospira group, a common finding in other studies too [8, 17].

In our study therefore comparative data from the biochemical tests and microbiological tests along with the clinical presentation and response to the treatment instituted were usually considered to clinch the diagnosis by the physicians.

South India being endemic for dengue, leptospirosis, malaria and other vector borne diseases and also with the incidence of other complicating factors such as alcohol dependence, diabetes mellitus and hepatic viruses slowly increasing, it becomes imperative to choose the tests to diagnose these diseases more accurately so as to support the clinical diagnosis and start early and appropriate management.

6. Conclusions

Though the addition of NS1 component to the diagnosis of Dengue infection has increased the chances of early detection, cross-reactions with other infectious diseases is still a problem. Reference labs with more specific tests are beyond the scope of hospitals in most Indian towns and cities and the cost could be inhibitive for most patients. More studies in these interpretive challenges will help to clinch the diagnosis for the clinicians and reduce the morbidity and mortality in endemic areas.

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