EVALUATION OF DIFFERENT LABORATORY PARAMETERS IN THE DIAGNOSIS OF LEPTOSPIROSIS

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HOW TO CITE THIS ARTICLE:

B Ananthi, Lata Sriram, G Sumathi. "Evaluation of different laboratory parameters in the diagnosis of leptospirosis". Journal of Evolution of Medical and Dental Sciences 2013; Vol2, Issue 48, December 02; Page: 9395-9403.

ABSTRACT: BACKGROUND: Leptospirosis is one among the many zoonotic diseases that have emerged and resulted in epidemics leading to significant morbidity and mortality in humans. Even though it is a potentially serious disease it can be treated. Early diagnosis and appropriate treatment will halt the progression of the disease and reduce the mortality and morbidity of leptospirosis. So awareness and knowledge about the clinical and laboratory profile of leptospirosis is valuable so that a quick clinical suspicion can be made out and diagnosed early for initiating appropriate treatment and for a better outcome. **MATERIALS AND METHODS:** Patients admitted with fever ≥ 5 days and of the age group eleven years and above were evaluated for leptospirosis by performing MSAT (Macroscopic slide agglutination test). 156 patients who were positive by MSAT were taken into the study and the period of study was one year. These patients were subjected to detailed evaluation and followed till death or discharge. The data of these patients were tabulated and analysed using simple statistical methods. MAT (Microscopic agglutination Test) was performed with all MSAT positive samples to determine the predominant serovar. **RESULTS:** Out of 156 patients 51.28% were males and the mean age group affected was 21 to 30 yrs. Predominant symptoms were fever (100%), coloured urine (62.82%), vomiting (48.07%) and headache (40.38%). On examination, icteric sclera was seen in 71.79%, and hepatomegaly in 32.69% of patients. The common serovars were *Leptospira icterohaemorrhagiae* (49.65%), *Leptospira australis* (28.64%), *Leptospira semaranga* (13.28%). Incidence of leptospirosis was seen throughout the year although the number of cases increased during monsoon season (August to February). Laboratory investigations revealed elevated ESR in 100%, thrombocytopenia in 50.64%, increased polymorphs in 66.66%, abnormal LFT (Liver Function Tests) in 71.79%, abnormal RFT (Renal Function Tests) in 26.92% and both abnormal LFT and RFT in 21.79% of patients. Two patients (1.28%) went into acute renal failure. Renal parameters were found to be increased (90.47%) more in thrombocytopenic patients. Pulmonary involvement was present only in 2.56% of patients. **DISCUSSION AND CONCLUSION:** In conclusion this study provides clinical and laboratory data describing the extent of the problem of leptospirosis which can be used for quick clinical judgement and suspicion of leptospirosis in future cases, particularly in communities which lack diagnostic facilities. *L. icterohaemorrhagiae* (49.65%) was the predominant serovar. There was no mortality observed in this study due to early recognition and intensive treatment, even in the severe form of disease.

INTRODUCTION: Leptospirosis is a zoonotic infection caused by the spirochaete belonging to the genus *Leptospira*. It affects humans worldwide, in both urban and rural areas and in temperate and tropical climates¹. Pathogenic leptospires live in the kidney of natural hosts, predominantly mammals and are excreted with the urine into the environment where they survive for several

months in humid, warm and slightly alkaline conditions. Humans are accidental hosts and are infected by contact with an environment contaminated by urine of shedder hosts such as rodents, cattle and dogs².

The route of infection is through abraded skin and mucus membranes. Pathogenic leptospires rapidly invade the bloodstream and causes disruption of the integrity of the cell membrane of the endothelial cells lining the blood vessels in all parts of the body. Capillary leakage and hemorrhages occur in all the organs and tissues, particularly the lungs, omentum and pericardium. Ischaemia from damage to blood vessels in the renal cortex leads to renal tubular necrosis, particularly of the proximal convoluted tubules. This may lead to renal failure that can be fatal. Liver cell necrosis caused by ischaemia and the destruction of hepatic architecture leads to the characteristic jaundice of the severe type of leptospirosis. Blood clotting mechanisms are affected by liver failure, aggravating the haemorrhagic tendencies³. Thus the clinical spectrum of leptospirosis is very wide with mild anicteric presentation at one end to severe leptospirosis with severe jaundice and multiple organ involvement on the other end⁴.

The clinical presentation of leptospirosis is a biphasic illness. The first phase of the disease is commonly referred to as the septicemic phase. It is characterised by fever, headache, myalgia, conjunctival congestion and a host of non-specific features that may include mild cough, lymphadenopathy, rash, anorexia, nausea, and vomiting. This phase is followed by a brief afebrile period of variable duration that, in turn, is followed by the immune phase of illness. The common organs involved during immune phase are the liver and kidneys. Both organ derangements are reversible. The severe form of leptospirosis, commonly known as "Weil's disease", is characterised by a fulminant course with rapid onset of hepatic and renal failure and high mortality⁵.

Its symptoms mimic many other diseases like dengue, viral hepatitis, meningitis, influenza and viral haemorrhagic fevers. Unless a high index of suspicion is maintained and laboratory assistance sought, clinical diagnosis is impossible, in a vast majority of cases, and leptospirosis will be missed in all but a few instances. In an endemic area, suspecting leptospirosis on clinical grounds should not be very difficult. It is therefore necessary to increase the awareness and the knowledge of leptospirosis, as it is a public health threat

Hence this study was conducted with the objective of evaluating different laboratory parameters in leptospirosis so that a quick clinical suspicion can be made out and a diagnosis arrived at early for initiating appropriate treatment and for a better outcome.

MATERIALS AND METHODS: This cross sectional study was done in Leptospirosis Research Cell, Institute of Microbiology, Madras Medical College, Chennai which included 378 patients who were admitted in the Government General Hospital, Chennai with fever of five days and more duration and belonging to the age group 11 years and above. Only patients with fever due to infectious disease were taken and those with fever associated with malignancies, autoimmune diseases were excluded from the study. Out of the 378 patients 156 were positive for (MSAT). Demographic data (age, sex), clinical signs and symptoms and the laboratory parameters of these 156 MSAT positive patients were recorded. Laboratory parameters include complete blood count, platelet count, ESR (Erythrocyte sedimentation rate), LFT (Liver Function Tests) and RFT (Renal Function Tests). Assessment of chest radiography was made for the presence and the type of infiltration. This study was reviewed and approved by Institutional Ethical committee.

MSAT (Macroscopic Slide Agglutination test)- This test is done using dense suspension of killed leptospires which is mixed with a drop of serum on a slide and rotated on a rotator (120rpm) for 4 minutes. It was then examined with naked eye for presence of agglutination. A two plus agglutination titre was considered significant.

MAT (Microscopic Agglutination Test) was performed on all MSAT positive samples to determine the predominant serovar. Eight serovars were included in antigenic preparation. *L. icterohaemorrhagiae*, *L. australis*, *L. autumnalis*, *L. hebdomadis*, *L. grippityphosa*, *L. louisiana*, *L. canicola* and *L. semaranga* were the serovars used. The antigen was prepared using standard methods. The highest dilution of serum antigen mixture which showed 50% agglutination was taken as the end point titre of the serum for that particular antigen. An initial titre >1:80 or a four-fold rise in titre of MAT was considered as positive.

RESULTS: The study population consisted of 156 patients; 80 men (51.28%) and 76 women (48.71%) with mean age group affected was 21-30yrs. Incidence of leptospirosis was seen throughout the year although the number of cases increased during monsoon season (August to February) [Fig 1]. The predominant symptoms were fever in all patients (100%), coloured urine in 98 (62.82%), vomiting in 75 (48.07%) and headache in 63 (40.38%) patients [Table 1]. The most common clinical findings were icterus in 112 (71.79%) and hepatomegaly in 51(32.69%) patients [Table 2]. Out of the 156 samples positive by MSAT, 143 were positive for MAT (91.66%). The common serovars were *Leptospira icterohaemorrhagiae* (49.65%), *Leptospira australis* (28.64%), *Leptospira semaranga* (13.28%), *Leptospira grippityphosa* (6.29%) [Table 3]. *Leptospira icterohaemorrhagiae* was found to be the predominant serovar in jaundice patients (66.96%) [Table 4]. Laboratory investigations revealed elevated ESR (100%) in all patients, thrombocytopenia in 79 (50.64%) patients. WBC was normal in 86 (55.12%), elevated in 44 (28.20%) and decreased in 26 (16.66%) patients. Abnormal LFT (Liver Function Tests) in 112 (71.79%) patients, abnormal RFT (Renal Function Tests) in 42 (26.92%) patients and both abnormal LFT and RFT in 34 (21.79%) patients [Table 5] was also observed. Two patients (1.28%) went into Acute Renal Failure. Both the cases recovered after appropriate treatment. Renal parameters were found to be increased (90.47%) more in thrombocytopenic patients [Table 8]. Elevated serum Total bilirubin level was seen in 112 (71.79%), raised SGOT (Serum Glutamic Oxalo acetic Transaminase) in 94 (60.25%), raised SGPT (Serum Glutamic Pyruvic Transaminase) in 91 (58.33%) and raised SAP (Serum Alkaline Phosphatase) in 90 (57.69%) patients [Table 9] was observed. X-ray chest revealed bilateral lower lobe opacity in 4 (2.56%) patients.

DISCUSSION AND CONCLUSION: Leptospirosis is considered as an emerging disease and this has led to an increased interest and demand for information, notably in developing countries. This infection is a public health threat due to its morbidity and mortality. The detailed knowledge of its clinical features and other laboratory parameters is necessary for early clinical diagnosis which is then confirmed by serological tests.

Out of 378 samples MSAT was positive in 156 samples (41.26%). MSAT is a rapid test used to identify if the person is infected or not. It detects the IgM antibodies. Sensitivity increases after a weeks time. Galton et al found that the Patoc strain with killed Ag used for MSAT was more sensitive in the second week of illness⁶. The usefulness of MSAT as a screening test has been evaluated by

Sumathi et al⁷. They have concluded that MSAT is a simple, quick and sensitive test which can be used as a screening test for laboratories in developing countries.

The highest number of cases was in the 21 – 30 year age group with a very slightly higher incidence in males. This might be due to increased outdoor activity, which increases chance of exposure to infection. Higher incidence of leptospirosis occurs following rainfall^{10, 11}. In this study, leptospirosis cases occurred throughout the year although the number increased during the rainy season (Fig 1). Polluted environment may be the cause which is an important epidemiological risk factor.

The predominant symptoms were fever (100%), followed by coloured urine (71.79%), vomiting (48.07%) and headache (40.38%) (Table 1). In Sritharan M et al study all the patients had fever with chills and myalgia (100%)¹². The most common signs observed in this study were jaundice (71.79%) and hepatomegaly (32.69%) (Table 2). This is in accordance with Margarita R et al study in which jaundice was present in 70.2% and hepatomegaly in 38.4% of patients⁸. But in Gancheva G et al study (2005) hepatosplenomegaly 100%, jaundice 75%, hypotension 33.33%, and haemorrhagic skin lesions 30.95% were the common signs¹³. The differences in distribution of major clinical features in this study might be due to the fact that the signs and symptoms keep varying from time to time as the infecting serovar also keeps changing with time.

The laboratory investigations in this study like ESR, WBC, Differential count were consistent with mild form of disease. Abnormal LFT, abnormal RFT and thrombocytopenia were associated with the severe form of the disease. There was significant inverse correlation between platelet count and serum creatinine in our study. This is in accordance with Ramon Peces' study¹⁷. Hence it was concluded that thrombocytopenia occurs frequently in renal insufficiency from leptospirosis and does not have a good prognosis. A high degree of suspicion, early recognition and intensive therapy could account for the complete recovery of these patients.

Only two cases (1.28%) out of 156 went into Acute Renal Failure. Both the cases after appropriate treatment recovered. No mortality was observed in this study. In Muthusethupathi MA et al (1994) study in Madras city during 1987-91 there were 120¹⁹ cases of Acute Renal Failure due to leptospirosis, but in the last two years there were only 15 cases of leptospiral ARF²⁰. In the present study there were only 2 cases. This indicates a decline in the severity of the disease and now more number of milder forms of disease occurs.

Total Serum bilirubin level is usually <20 mgm/dl in leptospirosis²¹. This fact correlates with the present study in which total serum bilirubin levels were less than 20 mgm/dl in all the cases of leptospirosis. In leptospirosis serum transaminases rarely exceed 200 IU/L in contrast to acute viral hepatitis²². This fact is in accordance with our study in which SGOT and SGPT were less than 200 IU/L in all the patients.

X-ray chest showed bilateral lower lobe opacity in 4 cases (2.56%) which indicate pulmonary involvement is rare in Chennai.

To conclude, recognition of leptospirosis in fever cases is important as accordingly appropriate antimicrobial agents can reduce its mortality and morbidity. Liver and the kidneys are the most common organs affected. Leptospirosis occurred throughout the year although the number of cases increased during monsoon season (August to February). *Leptospira icterohaemorrhagiae* (49.65%) was the predominant serovar. Due to early recognition and intensive treatment no mortality was observed in this study even in the severe form of disease.

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Symptoms	No of cases	Percentage
Fever	156	100
Coloured urine	98	62.82
Vomiting	75	48.07
Headache	63	40.38
Abdominal pain	54	34.61
Diarrhoea	53	33.97
Cough	53	33.97
Myalgia	50	32.05
Breathlessness	31	19.87
Pedal oedema	18	11.53
Conj. Suffusion	17	10.89
Joint pain	16	10.25
Dysuria	13	8.33
Hemoptysis	9	5.76
Giddiness	8	5.12
Oliguria	8	5.12
Restlessness	7	4.48
Haematuria	7	4.48
Altered sensorium	7	4.48
Skin rashes	5	3.20

Table 1: Symptoms of cases presenting with leptospirosis (n=156)

Signs	No. of cases	Percentage
Jaundice	112	71.79
Hepatomegaly	51	32.69
Hypertension	49	31.41
Anemia	32	20.51
Splenomegaly	30	19.23
Hypotension	3	1.92
Polycythemia	1	0.64

Table 2: Signs of cases presenting with leptospirosis (n=156)

Serovar	Positive titres			Total	Percentage
	1/80	1/160	1/320		
L. icterohaemorrhagiae	14	51	6	71	49.65
L. australis	10	25	6	41	28.67
L. semaranga	3	11	5	19	13.28
L. grippityphosa	6	3	-	9	6.29
L. canicola	1	2	-	3	1.92
L. louisiana	-	-	-	-	-

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L. autumnalis	-	-	-	-	-
L. hebdomadis	-	-	-	-	-

Table 3: Serovar distribution in leptospirosis positive cases (n=143)

*Out of 156 MSAT positive samples MAT was positive for 143

Serovar	No. of cases	Percentage
L. icterohaemorrhagiae	75	66.96
L. australis	24	21.42
L. semaranga	11	9.82
L. grippotyphosa	2	1.78
L. canicola	-	-
L. Louisiana	-	-
L. autumnalis	-	-
L. hebdomadis	-	-

Table 4: Serovar distribution in jaundice cases (n=112)

Investigations	No. of cases	Percentage
Elevation of ESR	156	100
Thrombocytopenia	79	50.64
Abnormal RFT	42	26.92
Abnormal LFT	112	71.79
Abnormal RFT and LFT	34	21.79

Table 5: Investigations in patients with leptospirosis (n=156)

Platelet Count cells/cmm	Range	No. of cases	Percentage
1,50,000 – 2,50,000	Normal	77	49.35
1,00,000 – 1,50,000	Decreased (Thrombocytopenia)	39	25.00
50,000 – 1,00,000		31	19.87
<50,000		9	5.77

Table 6: Platelet count in leptospirosis (n=156)

Renal parameters	Range		No. of cases	Percentage
Creatinine mgm/dl	<1.5	Normal	114	73.07
	1.5 – 2.0	Increased	26	16.67
	2.0 – 2.5		5	3.20
	2.5 – 3.0		1	0.64
	>3.0		10	6.41
Urea mgm/dl	20 - 40	Normal	114	73.07
	40 - 50	Increased	15	9.61
	50 - 100		16	10.26
	>100		11	7.05

Table 7: Renal function tests in leptospirosis (n=156)

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Parameters	Platelet count cells/cu. mm	No. of cases	Percentage
Increased Blood Urea and Serum Creatinine	1,50,000 – 2,50,000 (Normal range)	4	9.52
	<1,50,000 (Thrombocytopenia)	38	90.47

Table 8: Correlation of increased renal parameters with platelet count (n=42)

* Renal parameters were increased more in thrombocytopenic patients.

Liver parameters	Range		No. of cases	Percentage
Serum Bilirubin (Total) mgm/dl	<1	Normal	44	28.20
	1 – 5	Increased	76	48.72
	5 – 10		15	9.62
	>10		21	13.46
Serum Glutamic Oxalo acetic Transaminase (SGOT) IU/L	5 – 40	Normal	62	39.74
	41 – 60	Increased	30	19.23
	61 – 80		25	16.03
	81 – 100		14	8.97
	>100		25	16.03
Serum Glutamic Pyruvic Transaminase (SGPT) IU/L	7 – 35	Normal	65	41.67
	36 – 60	Increased	31	19.87
	61 – 80		30	19.23
	81 – 100		9	5.77
	>100		21	13.46
Serum Alkaline Phosphatase (SAP) IU/L	66	Normal	66	42.30
	10	Increased	10	6.41
	7		7	4.49
	73		73	46.79

Table 9: Liver Function Tests in leptospirosis (n=156)

Fig 1 Seasonal distribution of leptospirosis

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Date of Submission: 14/11/2013.
Date of Peer Review: 15/11/2013.
Date of Acceptance: 22/11/2013.
Date of Publishing: 28/11/2013