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SERUM CREATININE PHOSPHOKINASE [CPK] AN EARLY MARKER IN HUMAN LEPTOSPIROSIS

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Abstract- Introduction: Leptospirosis is zoonotic disease worldwide caused by genus Leptospira. It is characterized by non-specific clinical presentation with broad spectrum manifestations. Due to unreliable, unsophisticated diagnostic techniques, misdiagnosis is not uncommon & leads to endemic. **Aims and objective**: Evaluate hepatic dysfunction & correlation between CPK and other liver enzymes in Icteric & Anicteric group. **Material and Methods:** Total 170 subjects were examined by clinician & grouped in Icteric, Anicteric and healthy controls. Blood samples of all subjects were analyzed for Liver profile & IgM antibodies. The clinical finding includes myalgia, bilateral congectival suffusion, jaundice, abdominal cramps, albuminuria, hematuria & rashes on body were reported. **Results:** Male patients are more prone to Leptospira infection due to various occupational exposures. Serological tests comes positive until the second week, by that time organism implicate Liver and Renal function. Measurement of elevated CPK level and increased Faine's score will predict early diagnosis of disease. Morbidity & mortality can be minimized at an early stage of disease. **Conclusion:** Outcomes of this study will help clinicians to diagnose Leptospirosis at an early phase of disease and morbidity, mortality can be minimized.
Keywords: Leptospita-CPK-diagnosis

Introduction

Leptospirosis is zoonotic disease caused by genus Leptospira. The first spirochetes to be described as Leptospira interogans in 1907 by Stimons in renal tubules of Leptospira victim. Cause of disease is infection of spirochetes bacteria including pathogenic and saprophytic micro-organism [1,2]. Weil described the clinical manifestations like severe jaundice, fever and hemorrhage in 4 Japanese in 1886.[3] Leptospira disease is characterized by non-specific clinical presentation with broad spectrum manifestations.[1,4,5] Domestic population were generally the dead end host after transmission from animal contact.[4] In terms of pathological findings and clinical manifestations, liver is target of
spirochete infection [4, 5, 6, 7, 8] apart from kidney & lungs. Hepatic dysfunction in leptospirosis is usually mild, or severe and resolved eventually [2, 3, 9].

Serum alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) elevates moderately but serum creatinine phosphokinase (CPK) is usually raised drastically due to myalgia in Leptospirosis. [10] As per the WHO guidelines, Faine's criteria "myalgia score- 4 points out of 25 score", suggests a possible but unconfirmed diagnosis of leptospirosis [11].

Material and Methods

During period of April 2003 to March 2010, 170 patients including control group were tested. Majority of the patients recruited during flood situation in Mumbai during 2005 monsoon. Patients admitted in to the ICU & words with presumptive diagnosis of leptospirosis were included in the study. 49 anicteric, 36 icteric & 85 normal subjects were included in this study.

These patients were hospitalized in various tertiary care hospitals in south Mumbai (India). All subjects were grouped by applying WHO guidelines: Faine's criteria part A (clinical data) & part C (laboratory findings). [10, 12, 13]. None of the patient were alcoholics, nor suggestive of acute viral infection or diagnosed with hepatitis earlier. All patients were screened & confirmed by three different immunological assays & clinical findings.

Clinical parameters as per Faine's criteria & organ biochemistry analysis i.e. AST (normal 15-37 U/L), ALT (normal 30-65 U/L), ALP (normal 20 to 140 IU/L), GGTP (normal 30-65 U/L), CPK (normal male 35-232 U/L & female 21-215 U/L), Total Bilirubin (normal up to 1.0 mg%), Direct Bilirubin (normal 0-0.3 mg%), Total Protein (normal 6.4-8.gms%), Albumin (normal 3.4-5 gms%) & A/G ratio (normal 0.8-1.2) were analyzed. All methods

for analysis were approved by International Federation of Clinical Chemistry [IFCC].

Student 't" test were applied for all groups. Probability Value (P value) less than 0.05 were considered statistically significant. Liver biochemistry results which were always lacking as far as Leptospira diagnosis is concerned, is discussed in details.

Results

Leptospirosis is a zoonotic disease; onset was severe in all patients with fever (temp > 39 degree), chills & frontal headache. The clinical finding includes myalgia, bilateral Congectival suffusion, jaundice, abdominal cramps, albuminuria, hematuria & rashes on body. Eighty five cases of suspected Leptospirosis were admitted. Serological confirmation of disease is performed by three methods IgM ELISA, Dry Dot Micro- agglutination Assay (MAT) & Immunochromatography (ICT) Lateral Flow Assay.

P value less than 0.05 is significant & above 0.05 is non-significant. Statistical analysis of 85 suspected cases shows 62 (72.94%) male & 23 (27.06%) were female. Hence male patients dominated in both icteric & anicteric group. Biochemical parameters Creatinine Phosphokinase, Aspertate Transaminase, Total Proteins, A/G Ratio, Platelets and Creatinine shows significance "P" value in all study groups.

Leptospira infection has protean manifestations & frequently misdiagnosed. Epidemiological investigations of leptospirosis are often hampered by difficulty of making definitive diagnosis. Presence of organ dysfunction was significantly associated with mortality in icteric group. Raised Creatinine values reversed significantly after the treatment with antibiotics and IV fluids.

Discussion

Of the 85 patients admitted with presumptive diagnosis only 68.23% had serological evidence of disease. As per the Faine's criteria & WHO agreement; the initial diagnosis of Leptospira is still remain clinical & presumptive. Due to protean manifestations of disease there is clear correlation between complications & delay in treatment [12, 14].

Routinely available serological tools such as MAT (Agglutination test), ICT Lateral Flow Assay (Immuno-chromatography Technique) & ELISA do not show positive result immediate after onset of the symptoms. When patient is on treatment or a case of immunosuppressed syndromes, low titer of antibody level is also observed in early phase of disease.[14] Because of these reasons & pre-analytical factors measurement of IgM antibodies, qualitative or quantitative may not be reliable unless and until these results were not correlated with organ biochemistry and clinical data.

Gender analysis shows that the percentage of male patients is more than the female. Preponderance of male involvement in this study reflects the high risk of occupational exposure. Majority of them are from low socio-economical class, sugar cane farmers, sewer workers, rice field farmers as well as construction workers [15]. Large epidemics have been reported in the literature. In 1995, Nicargaun Leptospirosis epidemic was recorded due heavy flooding [16]. In 1996, Leptospira epidemic reported in Brazil [17] & in Peurto Rico [18].

Human infection occurs by the direct contact with urine or blood of infected animal. This can be transmitted through contaminated water, soil or vegetables. Liver involvement is common in hemorrhagic type of Leptospirosis. Though the elevated levels of Bilirubin, Total Protein, Albumin, enzymes ALT & AST are useful markers for prognosis [19]. Elevation of CPK level plays an important role in preliminary diagnosis. CPK very rapidly (>than 2000 IU/ML in few cases) as a single parameter in early stage of disease in icteric as well as anicteric group, rest all parameters including platelets shows normal results. Developments in critical care life supports the initial diagnosis still depends on high index of clinical suspicion, routinely available diagnostic tests being unreliable in early phase of disease [19].

Conclusion

It is concluded that ageing, high serum bilirubin levels [> 12 mg/dL], lack of reliable tests might be risk factors that increase end organ failure & ultimate mortality in leptospirosis. Fain's guidelines for diagnosis of leptospirosis has a great importance but misdiagnosis is not uncommon due to manifestations of the disease ranges from mild influenza like symptoms to severe renal, liver impairment & hemorrhages.

An increased level of all liver biochemistry parameters is demonstrated only after second week of onset of disease. In the initial phase of disease only CPK shown increased. By measurement of Faine's score & CPK level, one can presume the preliminary diagnosis of leptospirosis. If we wait to report positive results of IgM ELISA, spirochetes may damage end organs. Absolute damage to Kidney or Liver is irreversible. To avoid end organ failure screening of disease is possible at an early stage by measuring CPK level. Raised CPK may be due to invasion of pathogen spirochetes into skeletal muscle that causes generalized myalgia in 99% of confirmed cases.

The CPK is a simple test that may provide diagnostic information in a jaundiced patient, particularly when characteristic manifestations of leptospirosis were absent. The pattern of elevated CPK levels with only moderate elevations in transaminase values in an acutely jaundiced patient should strongly suggestive of leptospirosis infection.

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Table 1-Clinical presentation of leptospirosis

Clinical Data	Icteric group[36]	Anicteric group[49]
Temp < 39 degree	16 [45.71%]	21 [43.75%]
Temp > 39 degree	19 [54.28%]	27 [56.25%]
Chills	26 [72.22%]	32 [65.30%]
Frontal Headache	22 [61.11%]	30 [61.22%]
Myalgia	35 [97.22%]	47 [95.91%]
Bilateral Congectival Suffusion	06 [16.66%]	09 [18.36%]
Abdominal pain	04 [11.11%]	05 [10.20%]
Rashes on body	06 [16.66%]	04 [08.16%]

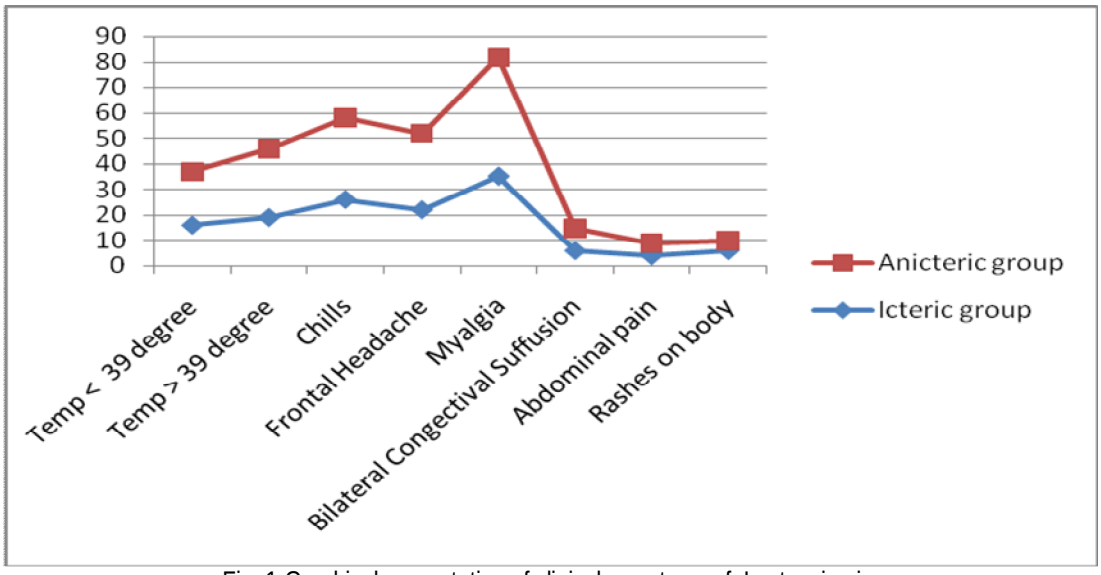

Fig. 1-Graphical presentation of clinical symptoms of Leptospirosis.

Table 2- Antigen- Antibody positive results of the patients with Leptospirosis.

Immunological Assay	Icteric group[36]	Anicteric group[49]
IgM Micro well ELISA	26 [72.22%]	32 [65.30%]
Dry Dot Agglutination	21 [58.33%]	26 [53.06%]
Lateral Flow ICT	23 [63.88%]	29 [59.18%]

Table 3- Biochemistry parameters of the four patients who died due to jaundice with high CPK values.

Sr.No	GGTP	AST	ALT	T.Bil	D.Bil	I.Bil	T.Prot	A/G	CPK
1	222	109	155	13.2	10.3	2.9	6.3	0.9	668
2	159	2752	5704	56.6	48.2	8.4	5.6	0.6	383
3	77	53	82	16.5	13.4	3.1	4.9	0.6	1070
4	164	71	73	12.6	10.8	1.8	5.7	0.8	1678

Table 4- Biochemical analysis of two recovered patient with high CPK from An-icteric group.

Sr.No	GGTP	AST	ALT	T.Bil	D.Bil	I.Bil	T.Prot	A/G	CPK
1	88	92	33	0.9	0.4	0.5	6.1	0.7	1846
2	89	83	45	0.7	0.3	0.4	6.7	0.8	5659

Table 5- Statistical significance of "P value" from An-icteric, Icteric group and Control group in various biochemical parameters

Sr No	**Parameter**	**I Vs AI**	**I Vs C**	**AI Vs C**
1	CPK	<0.05	<0.001	<0.001
2	AST	<0.02	<0.001	<0.02
3	ALT	>0.05	<0.02	>0.05
4	GGTP MALE	<0.05	<0.001	<0.02
5	GGTP FEMALE	>0.05	<0.002	<0.001
5	TOTAL BILIRUBIN	<0.001	<0.001	>0.05
6	TOTAL PROTEINS	<0.005	<0.001	<0.001
7	A/G RATIO	<0.005	<0.001	<0.005
8	PLATELET COUNT	>0.05	< 0.001	< 0.001
9	BLOOD CREATININE	<0.001	<0.001	>0.05

Keys; I-Icteric; AI- An-Icteric and C-Control.