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Evaluation of Polymerase Chain Reaction in Early Diagnosis of Leptospirosis

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A B S T R A C T

Keywords Polymerase chain reaction, Leptospirosis **Article Info** *Accepted:* 16 December 2017 *Available Online:* 10 January 2018	Leptospirosis is one of the most widespread worldwide zoonotic occupational disease which is widely prevalent in various states of India, especially in Tamil Nadu. The mortality in severe disease ranges from 5-40%. Early diagnosis and treatment with appropriate antibiotics contributes to a reduction in the mortality rate. Hence this study was done with the objective of evaluating the usefulness of PCR in early diagnosis of human leptospirosis. Blood samples collected from patients with clinical diagnosis of leptospirosis during the first week of illness and blood samples from normal healthy controls were included in the study. All samples and controls were subjected to serological assays- Latex agglutination test (LAT), Microscopic agglutination test (MAT), and Polymerase chain reaction (PCR) amplification using specific primers G1 and G2. Out of 75 samples from the patients with clinical diagnosis of leptospirosis, 78.7% were positive by PCR, while 54.6% were positive by MAT, and 50.6% by LAT. Among the 55 healthy controls, PCR & MAT were negative in all, whereas LAT was positive in 3.6%. PCR was found to be the most sensitive test for diagnosis of Leptospirosis during the initial phase of illness, as the sero-conversion usually takes a week to develop. PCR was observed with high specificity as well. Hence PCR is very useful in early diagnosis especially in fulminant cases of leptospirosis, where early treatment reduces the mortality rate.

Introduction

Leptospirosis is one of the most widespread worldwide zoonotic occupational disease with considerable medical and economical importance. It is widely prevalent in various states of India, especially in Tamil Nadu. The extremely protean clinical manifestations ranging from mild fever to severe life threatening illness with jaundice, renal failure, Pneumonia, hemorrhagic shock and meningitis make the diagnosis of leptospirosis difficult. The mortality rate in severe disease ranges from 5-40%. As leptospires are very sensitive to most of the antibiotics, early diagnosis and treatment with appropriate antibiotics have contributed to decrease the mortality. Conventional methods such as dark field microscopy, or culture isolation to detect the leptospires in clinical samples are either too unreliable or too slow to contribute to an

early diagnosis. Though serology forms the mainstay, diagnosis during the initial phase of illness is better achieved by molecular assays like PCR, as a single sample is sufficient unlike in serological assays, where paired sera are necessary for confirmation in many instances. Hence this study was done with the objective of evaluating the usefulness of PCR in early diagnosis of human leptospirosis.

Materials and Methods

This study was conducted in Government General Hospital, Madras Medical College, Chennai. The study group included 75 patients with clinical diagnosis of leptospirosis based on the modified Faine's criteria. The blood samples were collected during the first week of illness wherever possible, and the sera were subjected to antibody detection assays- Latex agglutination test (LAT), and Microscopic agglutination test (MAT). LAT was done for detection of IgM anti leptospira antibodies using a pooled leptospira antigen prepared from laboratory strains of *Leptospira interrogans* - serovars icterohemorrhagiae (M20), australis (ballico), autumnalis (Akiyami) and canicola (Hondutrecht IV) by sensitizing the latex beads (sigma), with the antigens. Microscopic agglutination test was performed for all the samples and controls using 8 live leptospira culture antigens (autumnalis, australis, canicola, javanica, Pomona, icterohemorrhagiae, sejroeand patoc) using standard microtiter method (Cole, *et al.,* Sulzer, Jones *et al.,*)

Polymerase chain reaction

PCR assays were performed using the primers G1 and G2 which were obtained from the Royal Tropical Institute. The PCR assay was carried out as per the method described by Grave Kamp *et al.,* The specific primers used in PCR for detection of leptospiral DNA are:
G1 – 5' CTG AAT CGC TGT ATA AAA GT 3'

G2 – 3' GGA AAT CAA ATG CTC GGA AG 5'

This is designed from the 5' end (nucleotides 1 – 20 G1) and 3' end (nucleotides 264 – 285 G2), representing the sequence of the recombinant plasmid pLIPs 60, which is selected from the genomic DNA library of *Leptospira interogans* strain RGA on the basis of strong hybridization signal with DNA from most leptospiral species. The primer was synthesized and obtained from NH Swellengrebel laboratory for Tropical Hygiene, Royal Tropical Institute, The Netherlands.

Results and Discussion

Blood samples from a total number of 75 patients with clinical diagnosis of Leptospirosis using modified Faine's criteria, were derived during the first week of illness and were included in the study for evaluation of various diagnostic procedures along with blood samples from 55 healthy controls. Males were the predominant group (78.6%) when compared with females (21.3%)

100% of the patients had fever with myalgia, followed by headache in (70.6%), conjunctival suffusion in 53.3%, pre-renal azotemia in 24%, meningism in 22.6%, jaundice 21.3%, albuminuria in 18.6%, and thrombocytopenia with rash in 10.6% (Table 1).

In the study conducted by M.A. Muthusethupathy *et al.,* (1997) fever, and myalgia were present in 100% of patients, renal impairment in 77% and conjunctival suffusion in 77%. Gastrointestinal symptoms were observed in 72% of patients in their study, whereas none of the patients presented with gastro intestinal symptoms.

Fig.1 Positive Amplification as seen as a single PCR product 285 bp for Leptospira
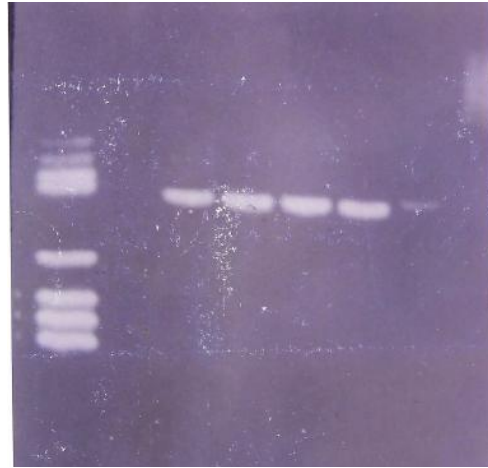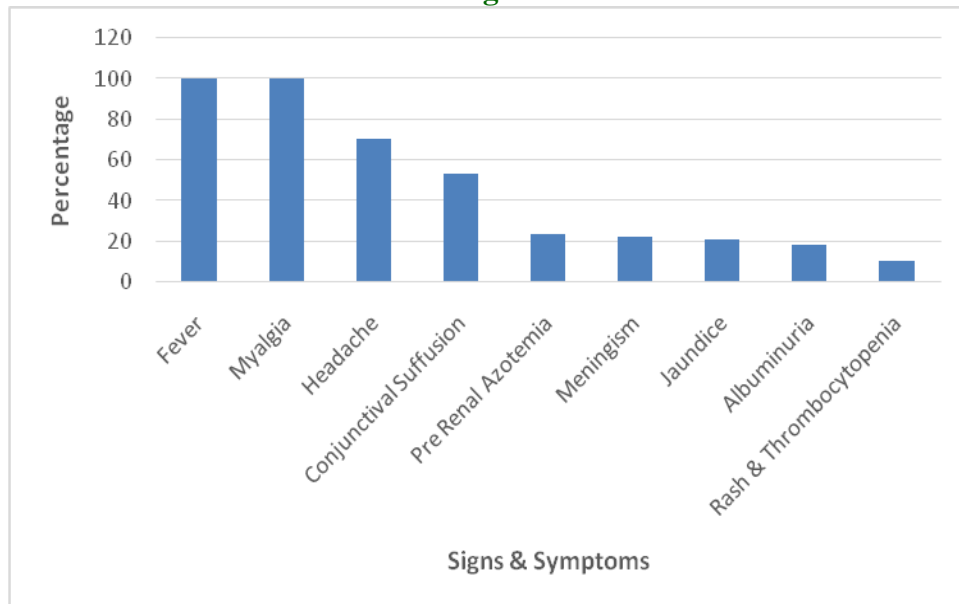

Fig.2

Table.1 Clinical signs and symptoms

Signs & Symptoms	Males	Females	Total
Fever	59	16	75 (100%)
Myalgia	59	16	75 (100%)
Headache	41	12	53 (70.6%)
Conjunctival Suffusion	31	9	40 (53.3%)
Pre Renal Azotemia	16	2	18 (24%)
Meningism	15	2	17 (22.6%)
Jaundice	15	1	16 (21.3%)
Albuminuria	14	0	14 (18.6%)
Rash and Thrombocytopenia	8	0	8 (10.6%)

Table.2 Sensitivity of various diagnostic procedures

Diagnostic Tests	Total no. of samples	No. of positives	Sensitivity %
LAT	75	38	50.6
PCR	75	59	78.6
MAT	75	41	54.6

Table.3 Comparison of PCR with LAT n=75

LAT	PCR		
	Positive	Negative	Total
Positive	38	0	38
Negative	21	16	37
Total	59	16	75

Table.4 Comparison of PCR with MAT n=75

MAT	PCR		
	Positive	Negative	Total
Positive	41	0	41
Negative	18	16	34
Total	59	16	75

Table.5 Species identification by MAT, n=41

Serovar	No. / %
Icterohaemorrhagiae	12 (29%)
Australis	14 (43%)
Non typable	15 (36%)
Total	41

The sensitivity of PCR, among the patients with clinical diagnosis of Leptospirosis is 78.6%, whereas, MAT and LAT showed sensitivities of 54.6% and 50.6% respectively (Table 2).

This study has shown an increased sensitivity of PCR when compared to the study done by Gravekamp *et al.,* (1995) and Brown *et al.,* (1994) where PCR showed a sensitivity of 50% and 69% respectively. Among the sera from 55 healthy controls, PCR and MAT did not test positive in any sample, whereas, 2 samples (3.6%) were positive by LAT.

Comparison of PCR and LAT results

LAT was positive in 38samples and negative in 37samples. PCR was positive in 21 out of 37 LAT negative samples (Table 3).

MAT was positive in 41 samples and 18 MAT negative samples were positive by PCR. 6 samples were negative by both PCR & MAT. Only in 5 patients a second serum sample was collected of which samples from 2 patients showed rise in titre (Table 4 and 5). This study was conducted with the aims and objective of evaluating usefulness of PCR in

early diagnosis of leptospirosis. Blood samples from 75 patients with clinical diagnosis of leptospirosis using modified Faine's criteria during first week of illness, and from 55 health controls were subjected to PCR, LAT and MAT. PCR showed a higher sensitivity of 78.6%, and MAT and LAT, 54.6% and 50.6% respectively. Both PCR and MAT were negative in all healthy controls samples. PCR contributes to the early diagnosis of Leptospirosis and make a useful tool especially in fulminant cases.

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