ORIGINAL ARTICLE

# Relevance of Polymerase Chain Reaction in Early Diagnosis of Leptospirosis

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## Abstract

**Aim and background:** Leptospirosis is common in India, especially in the southern states. Mortality is high among untreated cases. Diagnosis of leptospirosis remains a challenge in India as polymerase chain reaction (PCR), which is more sensitive than Immunoglobulin M (IgM) is not widely available. This study aimed to find out the difference in diagnostic yield with PCR and IgM in early leptospirosis.

**Materials and methods:** This retrospective, single-center study included 67 adults with laboratory-confirmed leptospirosis (IgM, PCR, or both) who presented within 7 days of symptom onset and were admitted to the intensive care unit (ICU). The difference in the diagnostic yield with PCR and IgM ELISA was studied.

**Results:** About 77.6% of the patients tested positive by PCR and 55.2% tested positive by IgM. There was a statistically significant difference in the detection of leptospirosis by PCR and IgM ($p$-value = 0.036). In the subgroup of patients who presented within 3 days of onset of symptoms, PCR positivity was 90.32% whereas IgM positivity was only 25.8%.

**Conclusion:** Our study showed that the sensitivity of leptospira PCR is significantly higher than IgM in the first week of illness. It also showed that among the subset of patients who died, a majority were detected only by PCR. Since PCR is not widely available, leptospirosis remains underdiagnosed and mortality from the same is underestimated. Polymerase chain reaction, if routinely done along with IgM for all suspected cases of leptospirosis that present within the first week of illness helps in prompt diagnosis and treatment.

**Keywords:** Intensive care unit patients, Immunoglobulin M, Leptospirosis, Polymerase chain reaction, Serology.

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## Highlights

Leptospirosis is a common cause of acute febrile illness in tropical regions. The commonly used diagnostic test is the detection of immunoglobulin M (IgM) antibodies by ELISA but this can miss early cases which can be mostly picked up by molecular methods. This study shows the importance of PCR in prompt and accurate diagnosis in the early phase of the disease.

## Introduction

Leptospirosis is commonly found in tropical regions with frequent rains and flooding. Farming and water sports pose a risk of exposure to leptospirosis. Another major etiological factor among people living in poor socioeconomic conditions is contact with stagnant water contaminated with rat urine.[1–3] The most important route of transmission is by contact of mucous membranes or abrasions in the skin with urine or tissues of infected rodents or soil contaminated with these.

In South Asia, it often spreads during the rainy season and post-flooding. In India, the disease outbreaks are most common in the southern part (25.6%), followed by northern, western, eastern and central India respectively.[4] Leptospirosis is a major public health concern in Kerala due to its epidemic potential. If left untreated, it results in high mortality among humans. Most cases are asymptomatic or mild. Symptomatic cases account for less than 15% of total infections.[5,6] Prompt and accurate laboratory diagnosis is crucial in view of the potential severity of the disease and the difficulty in diagnosing the infection clinically.[7] According to the data from the WHO, approximately 1.03 million persons are affected globally every year resulting in 58,900 deaths.[5,8] According to a systematic review, the morbidity and mortality due to leptospirosis is very high but poorly reported in South and Southeast Asian regions.[5] Mortality was found to be higher in older patients and in those with liver (19.1%) or kidney (12.1%) involvement.[7]

The timely laboratory diagnosis of leptospirosis is a formidable challenge. Various methods are available for the diagnosis of leptospirosis which include point-of-care tests, serological tests (IgM ELISA), hemagglutination tests, and microscopic agglutination test (MAT). Molecular diagnostics is a sensitive method for leptospira detection in acute care settings.[9] Introduction of polymerase chain reaction (PCR) has greatly helped in the diagnosis of leptospirosis during the early days of infection.[10,11] During early illness, it is the test of choice in terms of sensitivity and specificity which can aid in rapid diagnosis. However, the need for highly sophisticated equipment and skilled technicians is a major drawback for this test in low-income countries.[12] Due to these reasons, molecular- testing

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is not routinely available in many parts of India leading to gross underdiagnosis of leptospirosis cases.

After the first week, detection of specific IgM antibodies by serology is a reliable test for diagnosis. The negative predictive values of IgM and PCR are very low in the first and second week respectively.[12] Therefore, if PCR is used in conjunction with IgM, it helps in rapid and accurate diagnosis which is crucial for initiating proper and timely management.[13] The aim of this study was to find out the difference in diagnostic yield using IgM alone and when PCR was done along with IgM in suspected leptospirosis cases who presented during the first week of symptom onset.

## Materials and Methods

This retrospective study was conducted at a tertiary care center in central Kerala. Leptospirosis was suspected in adult patients presenting with clinical features as per the WHO case definition of leptospirosis.[14] Patients who presented with fever, chills, conjunctival suffusion, headache, myalgia, oliguria, and jaundice were suspected to have leptospirosis. As per our existing departmental protocol, both IgM and PCR were done in blood samples of all suspected cases of leptospirosis who presented within the first week of symptom onset.

All 67 such laboratory-confirmed (IgM, PCR, or both) leptospirosis cases who were admitted to ICU under the Critical Care Medicine department from October 2021 to July 2023 were included in the study.

### Test Methods

*Leptospira IgM ELISA (Abbott Diagnostics Korea Inc.)*

Qualitative detection of IgM antibodies to leptospira was performed using serum samples. The manufacturer's instructions were followed. For each test run, controls (both positive and negative) and calibrators were kept.[11] Panbio units and above were taken as positive and 9 or below Panbio units were taken as negative.

*Leptospira PCR (Truenat LTS-Molbio Diagnostics Private Limited)*

Chip-based real-time PCR test was used for the detection of leptospira in blood samples. Viral load was reported in terms of cycle threshold (Ct) which indicates the number of amplification cycles required for the signal to cross the threshold. The Ct value is inversely proportional to the target nucleic acid in the sample. At the end of the PCR cycle, positive results were displayed as "detected" and negatives as "not detected". If internal positive control (IPC) got amplified, the run was considered to be valid.

### Statistical Test

The data was statistically analyzed using IBM SPSS version 20.0 software and categorical variables were expressed as percentages and frequency. The statistical significance of the difference in the leptospirosis test positivity by PCR and IgM methods was tested using McNemar's Chi-square test.

Measures of agreement were computed by κ statistic. A *p*-value of $< 0.05$ was considered statistically significant.

## Results

Out of 67 laboratory-confirmed patients with leptospirosis (Table 1), 52 tested positive for PCR (77.6%) whereas 37 were positive for IgM (55.2%). In the subgroup of 31 patients who presented within 3 days of onset of symptoms, PCR was positive in 28 (90.32%), whereas IgM was positive only in 8 (25.8%). This showed that in patients who presented within 3 days, if only IgM was done, only around 25% would have been picked up and the remaining 75% would have been missed. On the other hand, PCR alone would have picked up around 90% during the same period. When the data of 55 patients who presented within 5 days of symptom onset was analyzed, PCR was found to be positive in 45 (81.82%) and IgM was positive in 25 (45.45%). This shows that diagnosis would have been missed in a significant number, had PCR not been done.

**Table 1:** Comparison of test positivity with PCR and IgM ($n = 67$)

| *Test positivity* | *Within 7 days* | *Within 5 days* | *Within 3 days* |
|---|---|---|---|
| PCR | 52/67 | 45/55 | 28/31 |
| IgM | 37/67 | 25/55 | 8/31 |

**Table 2:** Statistical significance of PCR

| | *IgM* | | | |
|---|---|---|---|---|
| *PCR* | *Positive* | *Negative* | *p-value* | *κ-statistic* |
| Positive | 22 | 30 | 0.036 | –0.426 |
| Negative | 15 | 0 | | |

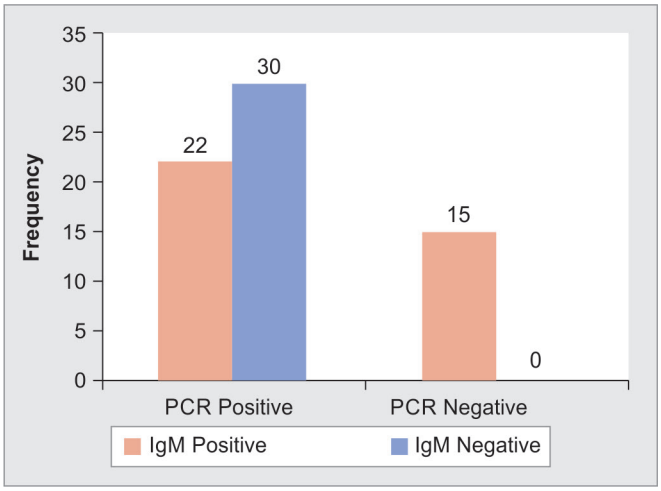


**Fig. 1:** Comparison of leptospirosis test positivity between PCR and IgM

Table 2 and Figure 1 present the results of a hypothetical reliability study of assessments of leptospirosis on two tests by a single examiner. The assessment categories were "positive" and "negative". The PCR method identified 52 positives and the IgM method identified 37 positives. A statistically significant difference in the assessments of leptospirosis by PCR method and IgM method (*p*-value = 0.036) was noted. The value of κ measures of agreement was –0.426 indicating there was "disagreement" between the two tests.

When patients were divided into 3 mutually exclusive groups based on the onset of symptoms at presentation, it was seen that the diagnostic yield of PCR was very high (90.625%) in the first 3 days which oppressively fell and reached 58.33% by the end of the first week. The pattern was the opposite in the case of IgM with a very low diagnostic yield in the first 3 days (25%) which progressively increased and reached 100% by the end of the first week (Table 3, Fig. 2).

**Table 3:** Change in test positivity during the first week of symptom onset

| *Test positivity* | *1–3 days* | *4–5 days* | *6–7 days* |
|---|---|---|---|
| PCR | 29/32 (90.625%) | 16/23 (69.565%) | 7/12 (58.33%) |
| IgM | 8/32 (25%) | 17/23 (73.915) | 12/12 (100%) |

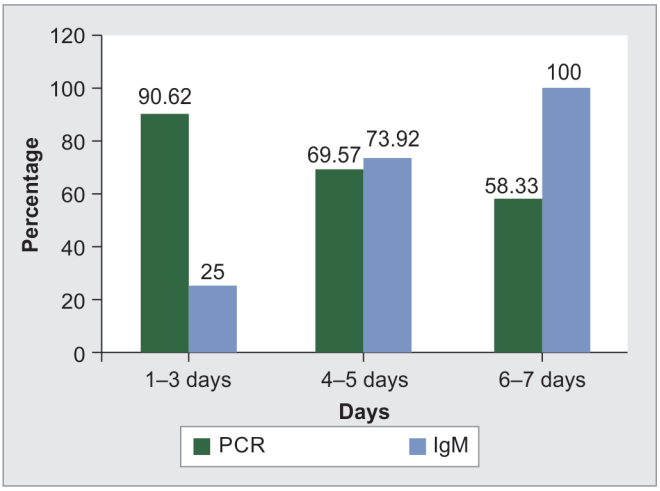


**Fig. 2:** Leptospirosis change in test positivity over the first week of illness

Out of 67 cases of leptospirosis included in this study, 9 persons died. All of them presented within 5 days of symptom onset (median: 3.33 days). Out of the 9, six were negative for IgM leptospira serology (66.67%) and were picked up solely by the PCR test. All except one out of the 6 died within 1 day of hospitalization.

Males were affected more than females (40/67, 68.66%). From 41 to 60 age-group was more commonly infected by leptospira (30/67, 44.77%) compared to <40 and >60 age-groups.

## Discussion

Leptospirosis is an acute febrile disease widely seen in areas with warm and humid climates, such as India. The illness is often underdiagnosed since growing these organisms in the laboratory is difficult and the appearance of detectable antibodies may take time.[15] Culturing leptospira from laboratory samples is time-consuming and cumbersome because leptospirosis require special media for growth and they are slow growers.[16,17] Polymerase chain reaction positivity peaks soon after symptom onset and hence PCR assay can be extremely useful as a method of diagnosis early on.[11,18,19] In leptospirosis, IgM antibodies begin to appear only by the end of the first week and therefore the sensitivity of these tests in early stages of illness is very low.[11,18,19] Therefore, in the first week of illness, a significant number of cases will be missed with IgM ELISA whereas a majority can be picked up with PCR. Hence, testing protocols using both PCR and IgM ELISA will improve diagnostic yield significantly.[20]

Out of a total of 67 laboratory-confirmed patients with leptospirosis included in our study, PCR positivity was 77.6% (52/67) whereas IgM positivity was only 55.2% (37/67). During the first 3 days, PCR positivity was found to be significantly high whereas that of IgM was very low. In our study, PCR has a sensitivity of 90.6% during the first 3 days of symptom onset. By the end of the first week, PCR positivity was observed to decrease. It was 55.83% in patients who presented on day 6 and 7 of symptom onset. IgM positivity followed the opposite trend with a positivity of 25% in the first 3 days of symptom onset. IgM positivity was shown to increase by the end of the first week reaching 100% on days 6 and 7 of illness. Diagnosis of leptospirosis is highly likely to be missed in the great majority of patients who develop MODS and die in the initial few days. In our study, a total of 9 patients died, of which 6 (66.67%) cases were detected only by PCR. This means that among non-survivors, the diagnosis would have been missed in a majority (66.7%). All of them died within 48 hours of presentation to our ED and would have remained undiagnosed if PCR was not done as they did not survive long enough for the IgM to become positive. This data suggests that deaths due to leptospirosis will be grossly underestimated unless PCR is done in all suspected cases who present early. Hence, to improve the diagnostic yield, the PCR test facility needs to be increased.

## Summary and Conclusion

Leptospirosis is a zoonotic disease with a global presence. Leptospires are bacteria belonging to the class Spirochaetes. It presents a range of clinical manifestations and the disease has high morbidity and mortality. Early diagnosis of critically ill patients poses a grave challenge as the pick rate of IgM leptospira, which is the most commonly used diagnostic test is low in the first week of illness. Technological advancements such as molecular diagnostics (PCR) have given a cutting edge to the early detection of leptospirosis cases. Our study showed that PCR positivity was 77.6% whereas IgM positivity was only 55.2% among leptospirosis cases who presented within the first week of symptom onset. However, PCR test positivity was 90.62% whereas IgM positivity was only 25% in those who presented within 3 days of symptom onset. Our study indicates that the number of diagnosed cases of leptospirosis is likely to be grossly wrong given the fact that the PCR test facility is not widely available and hence is being tested among only very few suspected patients.

In the subgroup of non-survivors, 66.67% were diagnosed with only PCR. All of them died within 1 day of hospitalization. This shows that in the sickest patients who died within a few days of symptom onset, the diagnosis of leptospirosis would have been missed in a majority since they did not survive long enough for the IgM to turn positive. It indicates that the mortality rate from leptospirosis would be significantly higher than what is available as per the current data since most centers are performing only IgM for the diagnosis of leptospirosis. This study suggests that patients presenting during the first week of onset of symptoms with clinical suspicion of leptospirosis should be tested for both IgM and PCR to increase the diagnostic yield.

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