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URINE PCR: A USEFUL DIAGNOSTIC TOOL DURING THE ACUTE PHASE OF LEPTOSPIROSIS?

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ABSTRACT

Leptospirosis is associated with high mortality if early appropriate treatment is not provided. Blood Polymerase chain reaction (PCR) is a highly sensitive test in the early phase of illness. This prospective study aimed to find out the usefulness of urine PCR in diagnosing leptospirosis in the early phase of illness. 50 adults admitted to the Intensive Care Unit (ICU) within 7 days of onset of symptoms with suspected leptospirosis were enrolled. Blood PCR, urine PCR and IgM were tested. 21 patients were tested positive by one or more of the above-mentioned tests. The diagnostic yield of urine PCR in comparison to blood PCR was studied. The statistical analysis of the test results obtained by blood PCR and urine PCR was done using McNemar's Chi-square test. It was found that 21 patients were tested positive for Leptospirosis by blood PCR, Urine PCR, or IgM or a combination of more than one of the above tests. Blood PCR was positive in 76.2%, urine PCR in 66.7% and IgM in 52.4%. It was also observed that among IgM-negative cases blood PCR positivity was 90% (9/10) and urine PCR positivity was 80% (8/10) and there was no statistically significant difference between blood PCR and urine PCR (p value=0.625). This study showed that when blood PCR was taken as the gold standard test for leptospirosis, the sensitivity of urine PCR was 81.25% in the first week of illness. Since urine PCR doesn't require phlebotomy and the point-of-care machines can be transported anywhere, it has a huge potential as a diagnostic tool during suspected community outbreaks of leptospirosis.

KEYWORDS: Intensive Care Unit patients, Leptospirosis, Polymerase chain reaction.

INTRODUCTION

Leptospirosis is a major public health problem in tropical countries where rains and floods are commonplace. Approximately three-fourths of the world's leptospirosis cases and an equal share of mortality are reported from these countries.[1] Untreated cases result in high morbidity and mortality. Hence timely initiation of appropriate treatment is warranted. Agricultural laborers and those involved in water sports are at a high risk of exposure. Exposure to stagnant water contaminated with rat urine poses a high risk to people of poor socioeconomic strata.[2-4] Humans are infected typically via abrasions in skin or mucous membranes when they come in contact with urine or tissues of rodents infected or colonized with leptospires. Indirect contact with soil or water contaminated with infected urine of rodents also can cause infection.[2,5] A great majority of cases are asymptomatic and many are only mildly symptomatic.[1,6] Accurate and early laboratory diagnosis is vital, given the potential severity of the disease and the difficulty in making a clinical diagnosis.[7] According to the WHO, leptospirosis affects approximately a million persons globally and accounts for 60000 deaths per year.[1,8] It is a major public health concern in the Southern states of India , especially Kerala where outbreaks are common during the rainy season.[9] Mortality is found to be high in the elderly as well as in those with liver (19.1%) or renal (12.1%) involvement. In acute care settings molecular diagnostics is a reliable method for leptospira detection. [10] Polymerase chain reaction (PCR) is known to be extremely useful in diagnosing leptospirosis during the initial days of infection. [11-13] Rapid and accurate diagnosis can be achieved by doing PCR along with IgM in suspected cases.[14,15] Studies have proved that during the early acute phase of illness, blood PCR has good sensitivity and specificity compared to IgM.[15] However, this test is not commonplace in low-income countries where equipment and skilled manpower are limiting factors. [16] This study aimed to find out the usefulness of urine PCR in diagnosing Leptospirosis in the early phase of illness.

MATERIALS AND METHODS

This prospective study was conducted at a tertiary care referral hospital in Kochi, Kerala. Approval from the ethics committee was obtained (LISIE/IEC/June 2023/02). Leptospirosis was suspected in patients who presented with fever, chills, conjunctival suffusion, headache, myalgia, oliguria, and jaundice as per the case definition laid down by the WHO.[17] As per the existing departmental protocol, blood samples were sent for both IgM and PCR from all suspected cases of leptospirosis who presented within the first week of onset of symptoms. Patients above 18 years of age admitted to the ICU under the Critical Care Medicine department from 17 July 2023 to 31 July 2024 with suspected Leptospirosis were included in the study. Informed consents were obtained from patients/surrogates. Urine samples for PCR were collected by the Microbiology laboratory whenever they received a request for blood PCR. Urine samples were collected not later than 4 hours of collecting the blood sample for PCR.

Test Methods

Truenat LTS which is a Chip Based Real Time PCR was used for both sample types. 0.5 ml of serum and 1ml of centrifuged urine deposit of the same patient were transferred into two separate lysis buffer tubes so that the specimen was decontaminated and readied for nucleic acid extraction. 6 µl of each of these were then transferred to separate microtubes containing RT-PCR reagents and allowed to stand for 1 minute to get a clear supernatant. The same volume was then pipetted into two micro PCR chips and loaded onto the Truelab Quattro machine (Truenat LTS-Molbio Diagnostics Private Limited). Once the test is completed, results are displayed as detected and not detected. Among the detected cases, microbial load is expressed semi-quantitatively as high, medium, low, and very low based on the cycle threshold values.

RESULTS

50 adult patients were enrolled in our study. Among them, 21 were tested positive for Leptospirosis by blood PCR, Urine PCR, or IGM. Blood PCR was positive in 16/21(76.2%) whereas urine PCR was positive in 14/21(66.7%). IgM was positive in 11/21(52.4%) (Fig.1).

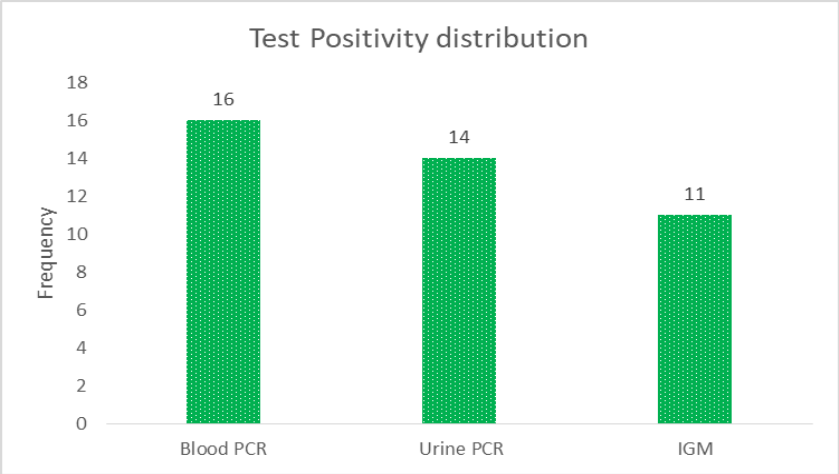

Fig. 1: Positivity distribution of different tests.

Among IgM-negative cases blood PCR positivity was 90% (9/10) and urine PCR positivity was 80% (8/10)(Table 1).

Table 1: Distribution of Polymerase chain reaction (PCR) positivity among IgM negative cases (n=10).

Test positivity	Frequency	Percentage
Blood PCR	9	90
Urine PCR	8	80

Though urine PCR positivity was only 60% among blood PCR-positive cases in the first 3 days of illness, it was found to be 100% by the end of the first week (Fig. 2).

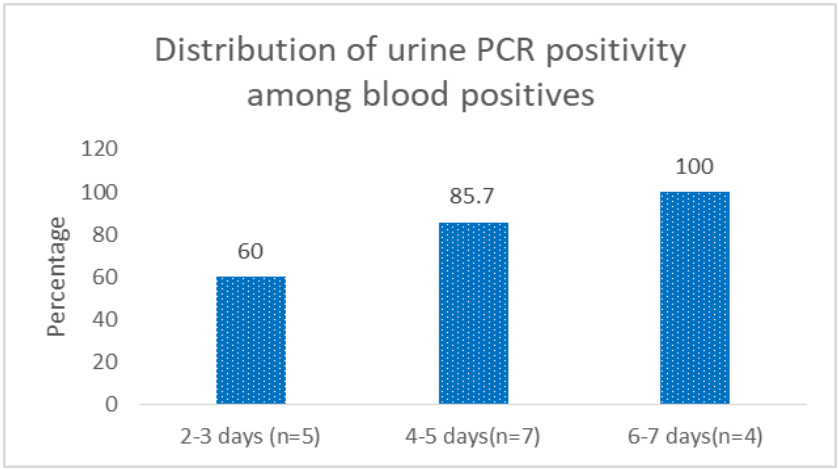

Fig. 2: Distribution of urine Polymerase chain reaction (PCR) positivity among blood PCR positives.

Among 16 blood PCR-positive patients, 13 were positive by urine PCR as well. There was no statistically significant difference between blood PCR and urine PCR (p value=0.625) in the diagnosis of leptospirosis in the acute phase of illness. Considering blood PCR as the gold standard for diagnosis of leptospirosis in the first week of illness, urine PCR

was found to have 81.25% sensitivity, 80% specificity, 92.9% Positive predictive value, 57.1% Negative predictive value and 80.6% accuracy. The statistical significance of the difference in the test results with blood PCR and urine PCR was tested using McNemar's Chi-square test.

DISCUSSION

Leptospirosis is widely seen in tropical regions with frequent rains and floods such as India. Antibodies reach detectable levels only by the end of the first week of illness.[18] Culturing leptospira requires special media and is a time-consuming process as the organisms are slow growers.[19,20] Due to these reasons, the illness often remains underdiagnosed. Blood-PCR positivity peaks during the initial days of illness, but IgM antibodies become detectable only by the end of the first week .[12, 21,22] Diagnostic yield during the early acute phase of illness will be significantly better if testing protocol includes both PCR and IgM.[23] A recent study by the same authors has found a statistically significant difference between blood PCR and IgM positivity in the first week of illness, especially in the first 3 days.[15] A study from Japan found that PCR positivity in urine lags behind blood PCR positivity by a few days.[13] Leptospires can be detected in urine when the immune system starts to produce antibodies and clear Leptospira from the blood. [24] A study which analyzed the kinetics of disease progression based on PCR positivity in paired samples (blood and urine) suggested that blood positive/ urine negative patients represent those in incubation period or early days of infection, whereas patients positive for both serum and urine represent early dissemination and kidney colonization phase.[25] Urine positive/ blood negative patients are in the late phase of illness with urinary excretion of leptospires. This happens approximately one week after disease onset.[26] Our study showed that overall sensitivity of urine PCR is 81.25% in the acute phase of illness when blood PCR was taken as the gold standard. Sub-group analysis showed the sensitivity was low in the first 3 days (60%) which increased to 85.7% on day 4 and 5 and reached 100% by the end of one week.

CONCLUSION

Blood PCR is known to be a diagnostic test with good sensitivity in the acute phase of illness before the IgM titer starts to rise by the end of the first week. Urine PCR is not commonly used as a diagnostic tool for leptospirosis. Our study showed that the overall sensitivity of urine PCR is 81.25% compared to blood PCR in the first week of illness. In the sub-group analysis, though the sensitivity was only 60% in the first 3 days, it reached 85% by midweek and 100% by the end of the first week. Given that urine PCR doesn't require phlebotomy and can be tested by point-of-care machines that can be transported anywhere, it has a huge potential as a diagnostic tool during suspected community outbreaks of leptospirosis.

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