**Case Report**

**Leptospirosis in a case of non-Hodgkin lymphoma**

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**Abstract**

Leptospiral meningitis, which is an important feature of anicteric leptospirosis, is generally underdiagnosed. Serological tests are not very useful in diagnosis of leptospiral meningitis. Early detection by molecular tests such as PCR and prompt institution of therapy would be life-saving.


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**Introduction**

Leptospirosis is caused by pathogenic spirochetes of the genus *Leptospira* and remains a challenge for public health officials and researchers, presenting significantly high rates of morbidity and mortality [1]. Meningitis can be a significant feature of the clinical profile of leptospirosis, mainly in the milder, anicteric forms of the disease [2]. Meningeal involvement in leptospirosis is typically biphasic in nature [1]. During the septic phase, leptospires can be recovered from the cerebrospinal fluid (CSF) by culture or can be demonstrated by polymerase chain reaction (PCR). During the immunological phase, lymphocytic pleocytosis occurs, with total cell counts usually below 500/μl, and the diagnosis can be made through immunological tests. In the later phase, the CSF is characterized by protein levels between 50 and 300 mg/dl, and the glucose concentration is generally normal [3]. Leptospirosis may account for 10% of cases of aseptic meningitis and the incidence is greater in children than in adults [4]. The use of PCR can facilitate the diagnosis when the antibodies are absent or at low levels, and leptospires are observed in high numbers in the CSF.

Several factors may contribute to the underdiagnosis of leptospiral meningitis. Though clinicians are sensitized to diagnose a patient with characteristic clinical features, sometimes the diagnosis is missed because of atypical presentation, especially when associated with neurological manifestations. These patients are then empirically treated and following poor therapeutic response are referred to a tertiary centre, often in a declining state. Hence it is essential to be aware of these uncommon manifestations of leptospirosis, especially with neurological deficits.

With this background we retrospectively undertook this study to establish leptospiral etiology in cases of aseptic meningitis. One hundred CSF samples that were bacteriologically culture negative were taken for this study. DNA was extracted from the CSF samples using the method described by Romero *et al.* using 10-1 TE buffer [5]. The positive control used was a CSF sample spiked with *Leptospira interrogans* serovar Icterohaemorrhagiae. A PCR mixture without template DNA comprised the negative control. PCR was performed using pathogenic species-specific primers amplifying the 16srRNA gene which resulted in a 631bp fragment [6]. PCR was done in duplicate for each of the samples.

The amplification of DNA was performed in a total volume of 25 μl. The primers used were
5’ CTCTGCCGGGCGCTTAAAA 3’ and 5’ TTCACCGCTACACCTGGAA 3’.

The PCR profile was as follows: Denaturation of DNA at 94°C for 1 minute, annealing at 55°C for 1 to 2 minutes and extension for 2 minutes. Final extension of the amplified product was performed at 72°C for 10 minutes. A total of 35 cycles were carried out. The products were analyzed on 0.8% agarose gel.

Case report

Out of the 100 samples tested, positive amplification of leptospiral DNA was obtained in one case only. This was a case of a six-year-old male child who had come to the medicine department of our hospital with complaints of fever and convulsions, with swelling of left side of face and left testes. The patient was residing in Cuddalore, a small district near Puducherry in South India. One year ago he had undergone left orchidectomy in the Institute of Child Health in Chennai, Tamilnadu, South India. A biopsy was done and the report was suggestive of high-grade non-Hodgkin lymphoma (NHL), B cell type-Stage III. On investigations at the time of presentation, his hemoglobin was 12.5 gm%, total leucocyte count was 11 x 10^9/L, and platelet count was 300 x 10^9/L. His blood urea (24 mg%), serum creatinine (0.9 mg%), serum bilirubin (0.7 mg%), alkaline phosphatase (181 units/L) and electrolyte values (sodium 146 mmol/L and potassium 3.87 mmole/L) were within normal levels. Alanine aminotransferase and aspartate aminotransferase values were also within normal limits. General examination revealed a normal pulse rate (80/min) and blood pressure (110/75 mm of Hg). Cardiovascular, respiratory and abdomen examinations were normal. Cerebrospinal fluid examination revealed a raised protein level (280 mg%) and a normal glucose value (58 mg%) and no cellularity. CSF culture did not yield any bacterial growth. The patient was given amoxicillin syrup 125 mg/ml for one week and chemotherapy with cyclophosphamide 160 mg in 500 ml of normal saline for four weeks, after which radiotherapy was given. The patient was later discharged after completion of treatment.

The rapid detection of leptospires at an early stage may favorably influence the course of the disease. The detection of specific antileptospiral antibodies in the CSF is a standard laboratory procedure for establishing the diagnosis, although during the early stages of leptospirosis, serological tests of CSF may be negative. A sensitive, specific, and rapid method for the diagnosis of leptospirosis is important both for the clinician and the patient. There are several pitfalls associated with detecting and interpreting immune responses, so the use of PCR to detect the DNA of *Leptospira* spp. seems promising.

In the present case, fever with convulsions with an altered CSF protein level and a positive amplification of leptospiral DNA in CSF were suggestive of leptospirosis. The IgM ELISA and MAT tests to support the findings could not be performed as the CSF samples were screened retrospectively. Hence a definite relationship between the leptospiral infection and NHL was difficult to establish.

Nevertheless, this finding would surely be encouraging to take up further such studies for knowing the incidence of leptospiral meningitis.

References

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