

APPLICATION OF ANTI-LEPTOSPIRA ELISA-IgM FOR THE ETIOLOGIC ELUCIDATION OF MENINGITIS

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SUMMARY

Leptospirosis is one of the causes of meningitis, although its importance is not well known. In the present study we contributed to this knowledge by demonstrating specific IgM class anti-leptospira antibodies by the immunoenzymatic method ELISA in 14.6% of cerebrospinal fluid (CSF) samples from 171 patients with meningitis considered to be of indeterminate etiology. The frequencies of positivity were similar in cases with predominance of polymorphonuclear or lymphomononuclear leucocytes in the CSF. Age distribution showed a predominance of the 5 to 15 year age range (72%), and sex distribution showed a predominance of males (68%). The authors discuss the contribution of this method to the etiologic elucidation of meningitis.

KEYWORDS: Leptospirosis; Meningitis and ELISA-IgM.

INTRODUCTION

Meningitis are affections involving the central nervous system that cause severe signs and symptoms, with an often lethal outcome or irreversible sequelae. The etiologic diagnosis is important for the institution of treatment and also for prophylactic measures and epidemiologic surveillance measures. Etiologic elucidation is not always possible due to several factors such as previous indiscriminate use of antimicrobial drugs and lack of sensitive laboratory tests capable of detecting all etiologic agents.

Among the etiologic agents that can cause meningitis are leptospire, as first reported by LAUBRY & PARVU¹⁵. In some cases, this happens relatively frequently where other mild presentations are not diagnosed, the leptospiral infection may present as meningitis with headache, fever, photophobia and vomiting and signs of meningeal irritation¹². The objective of the present study was to evaluate the presence of anti-

leptospira IgM antibodies in the cerebrospinal (CSF) fluid of patients with a diagnosis of meningitis of undetermined etiology seen at the Emilio Ribas Infectology Institute, São Paulo, Brazil, from January to July 1988 (summer and autumn months when rainfall is higher).

MATERIALS AND METHODS

CSF samples

CSF samples were divided into the following groups:

- positive control group: 5 samples from patients with the icterohemorrhagic form of leptospirosis, confirmed by microscopic agglutination test (MAT)⁷;
- Negative control group: 20 samples obtained during the induction of anesthesia from individuals with no clinical or epidemiologic history of leptospirosis, and negative by MAT were used. Twelve samples from pa-

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TABLE 1

Cases with a diagnosis of meningitis of indeterminate etiology according to age, sex and predominant leucocytes in cerebrospinal fluid (CSF), seen at the Emilio Ribas Infectology Institute, São Paulo, from January to July 1988.

Age (years)	Leucocytes in the CSF				
	Lymphomononuclear		Polymorphonuclear		Total
	Males	Females	Males	Females	
< 5	8	4	30	19	61 (35.7%)
5-15	21	6	15	10	52 (30.4%)
16-30	10	9	11	3	33 (19.3%)
> 30	2	4	12	7	25 (14.6%)
Total	41 (24.0%)	23 (13.4%)	68 (39.8%)	39 (22.8%)	171 (100%)

tients with meningitis caused by *Neisseria meningitidis* (n=2), *Streptococcus pneumoniae* (n=2), *Haemophilus* sp (n=2), *Cysticercus cellulosae* (n=2), *Mycobacterium tuberculosis* (n=2), and *Treponema pallidum* (n=2) (positive bacteriologic test)¹³ were also used as negative control group;

• samples from 171 patients with a diagnosis of indeterminate meningitis and with negative bacteriologic (bacterioscopy and culture on Müller-Hinton medium and chocolate agar) and immunologic (counterimmunoelectrophoresis for *Haemophilus influenzae*, *Neisseria meningitidis* and *Streptococcus pneumoniae*) tests. The 171 samples presented increased cellularity when examined cytologically, with 64 of them showing a predominance of lymphomononuclear leucocytes and 107 showing a predominance of polymorphonuclear leucocytes.

ELISA-IgM

The antigen was obtained from leptospira cultures of the serovars *brasiliensis*, *canicola*, *cynopteri*, *hebdomadis* and *icterohaemorrhagiae*. The leptospira were grown on EMJH medium (Difco Laboratories) at 28°C for 7 days, until the cell growth was about 5×10^7 bacteria ml⁻¹, and then frozen at -20°C for 7 days. After defrosting, they were washed twice and resuspended to 25% of the original volume in 0.02 M phosphate-buffered saline (PSB), pH 7.2. After disruption by sonicated at 20 KHz, for three periods, each of 3 min., the five sonicated leptospiral suspensions were mixed in equal proportions of protein contents (w/v) (antigen mixture), determined according to BRADFORD⁴ and used at final protein concentration of 3 µg/ml^{6,17}.

Elisa-IgM was applied by the technique of CAMARGO et al.⁶ using 50 µl of the reagents and CSF samples at 1:1 dilution. We used human anti-IgM antibody conjugated with alkaline phosphatase (Sigma

Chemical Co., St. Louis, MO, USA) and readings were taken with a Multiskan MCC plate spectrophotometer (Flow Laboratories). The cut-off was determined by the arithmetic mean plus 3 SD of the absorbances obtained for the CSF samples from patients with meningitis of determined etiology and from individuals with no clinical or epidemiologic antecedents.

RESULTS

The 171 patients with a diagnosis of meningitis of indeterminate etiology were distributed by predominance of leucocytes in the cytologic examination of CSF and by sex and age range (Table 1).

The reactivity threshold obtained with the anti-leptospira ELISA-IgM was 0.520 and the frequency of reactivity in the CSF samples with a predominance of lymphomononuclear and polymorphonuclear leucocytes was 14.1 and 14.9%, respectively.

Of the CSF samples from the 171 cases of indeterminate meningitis submitted to Elisa-IgM, 25 (14.6%) presented reactivity and were distributed as follows in terms of predominance of leucocytes upon cytologic examination: among the CSF samples with predominance of lymphomononuclear leucocytes, 8 from male patients aged 5 to 15 years and 1 from a female patient older than 30 years were reactive. Among the CSF samples with a predominance of polymorphonuclear leucocytes, 9 from male patients aged 5 to 15 years (n=6) and from male patients older than 30 years (n=3) and 7 from female patients aged 5 years (n=3) and 5 to 15 years (n=4) were reactive (Table 2).

DISCUSSION

The etiologic confirmation in cases of meningitis is of great importance and the clinical manifestations and cytologic examination of the CSF do not always permit its elucidation. Meningitis caused by leptospira at time

TABLE 2

Positive results to the ELISA-IgM test according to sex, age and predominance of leucocytes in the cerebrospinal fluid (CSF) in cases with a diagnosis of meningitis of indeterminate etiology seen at the Emilio Ribas Infectology Institute, São Paulo, from January to July 1988.

Age (years)	Positive ELISA-IgM test				
	Leucocytes in the CSF				
	Lymphomononuclears		Polymorphonuclears		Total
Males	Females	Males	Females		
< 5	-	-	-	3	3 (12%)
5-15	8	-	6	4	18 (72%)
16-30	-	-	-	-	-
> 30	-	1	3	-	4 (16%)
Total	8 (32%)	1 (4%)	9 (36%)	7 (28%)	25 (100%)

is not preceded or accompanied by jaundice³ and a predominance of polymorphonuclear or lymphomononuclear leucocytes may occur in the CSF depending on the phase and clinical form of the disease, a fact that greatly impairs diagnosis^{10,11}. WALCH-SORGDRAGER¹⁸ suggested that leptospiric meningitis, a name proposed by him, should be suspected in all cases of serous meningitis. This was based on 327 cases diagnosed bacteriologically and serologically at the Tropical Hygiene Institute of Amsterdam, and the same was observed by BEESON & HANKEY² at Grand Hospital, Atlanta, GA, USA.

Isolation of *Leptospira* sp from blood is possible only at the onset of the disease and only in specialized laboratories³; even so, indices are low, as observed in São Paulo^{5,8,9,16}. In most cases, the diagnosis of leptospiric meningitis is established from the result of microscopic serum agglutination¹ or CSF agglutination, usually with low (1:100) or even negative titers in the latter³.

Our group⁶ has standardized the ELISA for leptospirosis in the CSF, with results showing good specificity and sensitivity and permitting application of the test to the etiologic elucidation of meningitis by the detection of specific IgM antibodies that appear during the acute phase of the disease.

In the present study, the frequencies of reactivity were similar in both groups, demonstrating that the leucocyte subpopulation predominating in the CSF does not permit to formulate a diagnostic suspicion in leptospiric meningitis. This result emphasizes the importance of always submitting CSF samples from patients with indeterminate meningitis to the ELISA-IgM test.

The sex distribution of the positive results showed that the highest incidence occurred in CSF samples from male patients, following the same distribution of the various clinical forms of this disease¹². As to age range, the highest incidence of positive results occurred in the 5-15 year age group (72%), in contrast to the habitual incidence of the icterohemorrhagic form detected in serum, which is more frequent among young people and adults aged 15 to 44 years¹².

An outbreak of leptospirosis with meningoencephalitic manifestation has been reported among children aged 8 to 13 years¹⁴, coinciding with the age range in which the largest number of reactive results was observed in the present study.

The reduced information available in the specialized literature about leptospirosis in children, the data reporting a greater incidence of this disease among young adults, the report cited earlier¹⁴ and the present results permit us to suggest that ELISA-IgM should be better evaluated not only as a subsidy in the diagnostic elucidation of cases of meningitis of indeterminate etiology, but also as a contribution to a better understanding of the physiopathology and clinical manifestations of the disease by age range, and also to provide better epidemiologic information about the etiology of meningitis in Brazil.

RESUMO

Aplicação do ELISA-IgM anti-leptospira na elucidação etiológica das meningites.

A Leptospirose é uma das causas de meningite, embora sua importância seja pouco conhecida. O presente estudo contribui para este conhecimento ao demonstrar anticorpos específicos da classe IgM anti-

Leptospira pelo método imunoenzimático (ELISA), em 14,6% das amostras de líquido cefalorraquianos (LCR) de 171 pacientes com meningite considerada de etiologia indeterminada. As freqüências de positividade foram parecidas nos casos com predomínio no LCR de leucócitos polimorfonucleares ou linfomononucleares. A distribuição por idade mostrou predomínio na faixa etária entre 5 e 15 anos (72%) e por sexo o predomínio do masculino (68%). Os autores discutem a contribuição desse método na elucidação etiológica das meningites.

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Recebido para publicação em 19/10/1995

Aceito para publicação em 27/02/1996