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Original article

Enterovirus reverse transcriptase polymerase chain reaction assay in cerebrospinal fluid: An essential tool in meningitis management in childhood

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ABSTRACT

Background: Enteroviruses (EV) are the main aetiological agents of aseptic meningitis in children and a common cause of febrile illnesses in young infants in summer. A rapid diagnosis is essential to rule out other conditions. Real-time reverse transcriptase polymerase chain reaction (RT-PCR) assay performed in cerebrospinal fluid (CSF) has proved to be a very fast and useful tool.
Methods: We collected demographic, clinical and laboratory data of children (aged 11-years or younger) with EV RT-PCR (Cepheid® Xpert EV) positive in CSF from December 2007 to July 2010, to describe EV meningitis in children and to determine the role of this assay.
Results: We included 92 children (mean age 2.5 years), 32% of whom were neonates. There was no pleocytosis in the CSF of 18.5% (36% in newborn) of the patients, and 23 (25%) were discharged to home from the Emergency Room after the positive results. Length of hospital stay was 2 days (>2 years) versus 4.5 days in newborns ($P < 0.0001$). Antibiotic treatment was prescribed in 38% (75% <3 months), but in 40% of these, it was stopped after the positive results. Mean EV RT-PCR information time was 7 h (4–18 h). All children had a good clinical outcome.
Conclusions: EV RT-PCR assay in CSF has played an essential role in the management of children with EV meningitis, allowing earlier discharges and decreasing avoidable inappropriate antibiotic treatments. This test should be considered as part of the initial study of children with aseptic meningitis, especially during epidemic seasons.

Reacción en cadena de la polimerasa a tiempo real de Enterovirus en líquido cefalorraquídeo, una herramienta esencial para el estudio de meningitis en niños

Palabras clave:
Enterovirus
Meningitis aséptica
Infecciones víricas en neonatos

RESUMEN

Introducción: Los enterovirus (EV) son los principales agentes etiológicos de meningitis aséptica en niños, y una causa frecuente de síndrome febril en lactantes durante el verano. El diagnóstico rápido es esencial para descartar otras entidades. La reacción en cadena de la polimerasa en tiempo real (RT-PCR) realizada en líquido cefalorraquídeo (LCR) ha demostrado ser una herramienta rápida y útil.
Métodos: Se recogieron datos demográficos, clínicos y de laboratorio de los niños (<11 años) con RT-PCR a EV (Cepheid® Xpert EV) positiva en LCR desde diciembre de 2007 a julio de 2010 para describir las meningitis por EV en niños y conocer el papel de esta técnica.
Resultados: Incluimos a 92 niños (edad media 2,5 años), un 32% neonatos. El 18,5% (36% de los neonatos) no tenía pleocitosis en el LCR, 23 (25%) se fueron de alta desde la Sala de Urgencias tras el resultado positivo. La estancia hospitalaria fue de 2 días (>2 años) versus 4,5 días en neonatos ($p < 0,0001$). Se pautó tratamiento antibiótico en el 38% (75% <3 meses), pero en el 40% se suspendió tras el resultado positivo. El tiempo medio de información del resultado de RT-PCR a EV fue de 7 h (4-18 h). La evolución fue favorable en todos los casos.

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Conclusiones: La RT-PCR a EV en LCR ha desempeñado un papel esencial en el manejo de los niños con meningitis por EV, permitiendo altas más precoces y disminuyendo los tratamientos antibióticos inadecuados. Este test debería considerarse dentro del estudio inicial de los niños con meningitis aséptica, especialmente en los meses epidémicos.

Introduction

EV are the most important causative agents of aseptic meningitis in children.[1,2] These viruses are also associated with other diverse clinical syndromes as asymptomatic infection, fever in young infants, respiratory illness, gastroenteritis and severe neonatal sepsis-like disease.[3–8] EV meningitis in children are usually benign and require only symptomatic treatment.[1,3,4] Sometimes clinical presentation can be quite similar to other meningitis that require specific treatment, so earlier diagnosis helps to avoid additional investigations to rule out other aetiological agents, prevents unnecessary antibiotic treatment and decreases hospitalization.[9,10]

Classical diagnostic methods of EV meningitis were based on virus isolation, but viral cultures are not useful for treatment decisions because results take several days to weeks.[11–13] During the last two decades, real-time polymerase chain reaction (RT-PCR), performed in CSF has proved to be faster, more sensitive than viral culture and highly specific for the diagnosis of EV-meningitis.[13] Previous studies have described a significant impact of positive results in length of stay and duration of parenteral antibiotic therapy even in infants younger than 90 days.[10,14–17] Even, some authors have communicated a significant correlation between decreasing length of hospital stay and RT-PCR test turnaround time.[15]

In our institution, EV RT-PCR test in CSF is available from November 2007, so we conducted the present study with the aim to describe the epidemiological, clinical, and laboratory characteristics of EV meningitis in our paediatric population and to evaluate the role of EV RT-PCR in CSF for the diagnosis and management of this infection in our hospital.

Methods

Our hospital is a tertiary care medical centre serving a paediatric population of approximately 102,600 children aged less than 11 years.

From December 2007 to July 2010 we recorded, epidemiological and clinical features, laboratory results, hospital admission, antimicrobial treatment, length of hospital stay and outcome of all children (aged 11 years or younger) with EV RT-PCR positive in CSF. Blood and CSF bacteriological cultures were obtained in all patients, and also a urine sample was collected in neonates for bacterial culture. Informed consent was obtained in all cases. During this period a total of 361 samples of CSF (of patients younger than 11 years) were processed for bacterial culture and EV RT-PCR assay was performed in 194 of them.

Enteroviruses real-time reverse transcriptase polymerase chain reaction assay

The Cepheid® Xpert EV assay is a reverse transcription polymerase chain reaction (RT-PCR) using the GeneXpert® System for the presumptive qualitative detection of four species of EV (A, B, C, D) and the poliovirus (1, 2 and 3). It combines automated nucleic acid sample preparation, amplification and real-time detection of enteroviral RNA in about 2.5 h. This assay was designed to detect EV-RNA (enterovirus genome 5′ untranslated region [UTR] between nucleotide 452 and 596).[18] RT-PCR assay was performed in all CSF samples of neonates and young infants (aged 3 months or less) with fever without focus, and in CSF samples of children clinically suspected of having meningitis with absence of microorganisms on Gram stain.

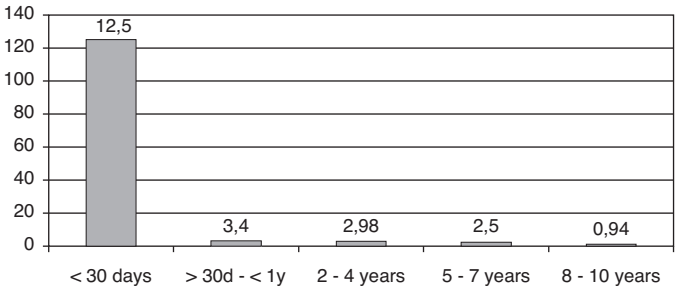

Fig. 1. Cumulative incidence of EV meningitis in each age group (number of cases/10,000 patients).

CSF pleocytosis was defined using previously published reference criteria for CSF white blood cell count as more than 20 cells/mm^3 for neonates and more than 10 cells/mm^3 for children older than 1 month.[19]

Statistics

The data were organized in a database. For the analysis, the study population was divided into 2 groups, patients aged 1 month or younger and those older than 1 month. All calculations were performed with statistical software SPSS version 18.0. Quantitative data are presented as means and range and qualitative data as the number of observations and percentages. Chi-square test was used to compare categorical variables and the Students *t*-test to compare continuous variables. The results were considered as statistically significant for two-sided *P* values of <0.05.

Results

We have studied 92 children aged 11 years or younger with EV RT-PCR positive in CSF. That represents 56% of patients (<11 years) with meningitis in our institution. The mean age was 2.5 years (5 days–10 years) and 32% (30 patients) were neonates (see Fig. 1). The majority (70%) of patients were male and 67% of newborn with EV meningitis had relatives (brothers and/or sisters) aged 3 years or younger. Though most cases (44 in June–July) presented during summer months many cases were also diagnosed during the fall (see Fig. 2).

The main clinical symptoms were fever (98%), irritability (40%) and respiratory symptoms (30%) in neonates and, fever (98%), vomits (88%) and headache (76%) in older patients. Neck stiffness was noted in all patients older than 2 years. The time of evolution of symptoms was less than 12 h in 74% of newborns versus 16% in older patients ($P < 0.001$). Clinical and laboratory results are summarized in Table 1.

CSF examination was performed in young infants <1 year (39 patients) as part of evaluation for fever without focus and in older patients to rule out meningitis. The mean number of CSF cell count was 254 cells/mm^3, and the mean protein and glucose levels were 55 mg/dl and 60 mg/dl respectively. Seventeen (18.5%) patients (36.6% of neonates) had no pleocytosis in CSF.