

Full Length Research Paper

Cerebrospinal fluid characteristics of PCR diagnosed enteroviral meningitis (EVM) in children

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Distinguishing bacterial from viral meningitis helps prevent unnecessary use of antibiotics and decreases the length of stay. The distinction may often be blurred by the typical textbook description of viral meningitis which states that mononuclear cells predominate in the cerebrospinal fluid (CSF). The aim of the work is to accurately describe the CSF response in a group of children with enteroviral meningitis proven by the gold standard, polymerase chain reaction (PCR). A cross sectional study was performed at Sheikh Khalifa Medical City (SKMC), Abu Dhabi, United Arab Emirates (UAE). We examined retrospectively all children (< 12 years as per hospital definition) who tested PCR positive for enterovirus in the CSF from January 2005 to January 2007. 53 patients with mean age (SD) of 74.8 months (37.8) were included. There was a statistically significant dominance in polymorphnuclear cells (PMN) in both the CSF (Chi Square statistic 28.78, $P < 0.001$) and serum, which persisted after 24 h with mononuclear cells and PMN equally represented. A correlation between CSF and serum PMN% was also detected (Pearson's $r = 0.447$, $P=0.001$, 95% CI = 0.196 to 0.643). Our peak season was in spring, there was a male predominance and the median (IQR) length of stay was 48 h (48 -72). The majority of children with aseptic meningitis had PMN predominance in both CSF and serum, which is not limited to the first 24 h. This finding differs from most standard textbook descriptions and may have relevance in using PMN counts to distinguish bacterial from viral meningitis.

Key words: Enterovirus, viral meningitis, PCR, polymorphnuclear cells, length of stay, children.

INTRODUCTION

Acute meningitis in children is predominantly aseptic and does not require specific treatment. However, meningitis has a bacterial origin in 4-6% of patients and carries a risk of death or severe sequelae (Dubos et al., 2008). Bacterial meningitis is usually suspected on the basis of the clinical presentation of the patient and the findings of purulence in the cerebrospinal fluid (CSF). The conventional diagnosis of enterovirus infections of the central nervous system relies on the recovery of the virus from the CSF or from a peripheral site (e.g. throat, stools) by cell culture. However, this technique is not very sensitive, especially for CSF specimens (due to low viral burden) and takes an average of 6 to 7 days to detect viral growth (Lina et al., 1996).

Distinguishing between bacterial and aseptic meningitis in children in the emergency department could contribute to limiting unnecessary antibiotic use and hospital admissions. The early predominance of polymorph-nuclear (PMN) cells in the CSF WBCs of patients who in the course of aseptic meningitis has been described. However, studies attempting to document the timing of transition from CSF PMN to mononuclear cells report conflicting results. Whereas some suggests that the transition occurs 8 to 24 h after presentation, others failed to find a correlation between the duration of symptoms and CSF PMN predominance (Bottner et al., 2002; Shah et al., 2006). Also, in certain conditions the cultures may remain negative if the disease is caused by fastidious and slowly growing microorganisms. In these situations molecular diagnostic method, including polymerase chain reaction (PCR) may provide a rapid and sensitive testing method for the diagnosis of enteroviral infections, which

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may expand the list of diseases attributable to this group of pathogens. PCR techniques have increasingly been used to amplify and detect microbial DNA in clinical samples (Ahmed et al., 1997).

We hypothesized that a predominance of PMNs in patients with aseptic meningitis is more common than previously documented and is not limited to the first 24 h of illness. This study was conducted with the primary aim of assessing the CSF characteristics in children with PCR proven enteroviral meningitis (EVM) and to examine the influence of the duration of illness on these findings.

MATERIALS AND METHODS

Study design and setting

A cross sectional study was performed at Sheikh Khalifa Medical City (SKMC), in Abu Dhabi which is the capital city of the United Arab Emirates. Our hospital is a 500 bed tertiary care institution with 75 pediatric inpatient beds. We examined retrospectively all children (< 12 years as per hospital definition), who tested positive for enterovirus by CSF PCR. A retrospective chart review was conducted of all patients less than 12 years of age hospitalized at SKMC with a diagnosis of EVM from January 2005 to January 2007. Patients were defined as having EVM if they had:

- 1-EV genome detected by CSF PCR
- 2-CSF pleocytosis (more than 5 WBCs/mm³) and
- 3-Negative CSF bacterial culture.

The only exclusion criteria were for the following reasons: age less than 30 days or macroscopically bloody taps (in both instances the CSF characteristics are markedly different), if they had a blood culture positive as well as EVM, and if they had an underlying immunodeficiency or neurological diagnosis. We did not exclude patients if they were on prior antibiotics because co-infection with both EV as well as a bacterium is very rare. Data collected for each patient included demographic information, duration of symptoms before presentation to the hospital, CSF parameters and haematologic parameters. The duration of symptoms was defined as number of hours between the onset of the first symptom noted by the parents or the patient and the performance of the lumbar puncture. PMN were considered predominant when the percentage of the neutrophils added to the juvenile forms was more than 50%.

Molecular genetics

For the detection of enteroviruses, RNA was extracted from 140 L of CSF with QIAamp Viral RNA Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. All specimens were reverse transcribed and PCR amplified in a single reaction followed by a semi-nested reamplification for those with a low or absent amplification signal. This testing strategy was implemented according to methods previously described (Watkins-Riedel et al., 2002).

In brief, for the first round LightCycler-PCR (1°LC-PCR) assay glass capillaries were loaded with 18 L master mix (1x LightCycler RNA Master HybProbe Buffer (Roche Diagnostics, Mannheim, Germany), 3.5 mM Mn(OAc)₂, 0.5 M primers E1 5'-CCC CTG AAT GCG GCT AAT CC-3' and E2 5'-CAA TTG TCA CCA TAA GCA GCC A-3', 0.25 M TaqMan probe EntTM1 5'-[6FAM-]CAC CCA CCA AAG TAG TCG GTT CC [TAMRA]-3') and 2 L sample RNA. The reactions were cycled in a LightCycler Instrument (Roche Diagnostics, Mannheim, Germany) through the following temperature program: 61°C for 20 min, 95°C for 30 s, 60 cycles of 95°C

for 3 s, 62°C for 10 s, 72°C for 12 s with a touch-down target of 60°C and a step size of 0.2°C per cycle.

The second round LightCycler-PCR (2°LC-PCR) assay contained 19 L master mix (1x PCR Buffer, 4 mM Mg²⁺, BSA 0.5 g/L (final concentration), 250 nM dNTP, 0.3 M primers CoxF 5'-GTA ACG GGC AAC TCT GCA GC-3' and PolF 5'-CGT AAC GCG CAA GTC TGT GG-3', 0.5 M primer EntR3 5'-ATT GTC ACC ATA AGC AGC CA-3', 0.25 M TaqMan probe EntTM1, 0.5 Units of Platinum Taq DNA Polymerase (Invitrogen, Carlsbad, California, USA)) and 1 L 1°LC-PCR product. The 2°LC-PCR temperature profile included the following steps: 95°C for 30 s, 50 cycles of 95°C for 5 s, 62°C for 10 s, 72°C for 10 s.

Each amplification run contained two positive controls, Armored RNA Quant. Enterovirus from Asuragen, Austin, Texas, USA, and a previously amplified positive cDNA sample in two dilutions (1:1000 and 1:10000). All primers and probes were synthesized by Metabion, Martinsried, Germany.

Statistical analysis

Descriptive characteristics are expressed as mean and standard deviation (SD), as well as median and the interquartile range (25P-75P). Correlations were performed by Pearson's product moment coefficient. Comparison of proportions was done by Chi Square testing. A *P* value < 0.05 was regarded as statistically significant. All hypotheses testing were two-tailed. Statistical analyses were performed using MedCalc for Windows, version 10.0.2.0 (MedCalc Software, Mariakerke, Belgium). This study was approved by the Institutional Review Board at Sheikh Khalifa Medical City.

RESULTS

The demographic details of our population with their CSF and haematologic characteristics are depicted in Table 1. We excluded 10 children as per our exclusion criteria (7 neonates and 3 with bloody CSF taps). There were 68.5% males and 31.5% females. The mean age (SD) was 74.8 months (37.8). Figure 1 depicts the seasonal variation in the EVM cases over the 24 month study period. The peak EVM season was during April whereas no cases were recorded in October for both years. The PMN and lymphocyte percent of both CSF and serum is shown in Table 1. Figure 2 shows the relationship between the serum and CSF of PMN's (Pearson's *r* = 0.447, *P* = 0.001, 95% CI = 0.196 to 0.643). In children who presented within the first 24 h of onset of symptoms, 77 % had PMN predominance in the CSF (i.e. CSF PMN > 50%) (Chi Square statistic 28.78, *P* < 0.001), whereas the cases presenting after 24 h were found with equal distribution of either PMN's or lymphocyte dominance (Figure 3). The median (25P-75P) length of stay was 48 h (48 -72). No patient had a repeat lumbar puncture. There was a 100% survival and no patients required subsequent readmission to hospital related to EVM.

DISCUSSION

Our data showed that PMNs dominate in the early and persist in the late phase in PCR proven EVM. The mean percent of PMNs among all our patients was 56.8,

Table 1. Summary statistics of enteroviral meningitis patients.

	N	Mean	SD	Median	#25-75P
Age (months)	53	74.8	37.8	73.3	50.5-99.9
Symptoms (hrs)	51	55.8	67.1	24.0	12.0-72.0
CSF protein (g/l)	53	0.4	0.1	0.3	0.2-0.5
Glucose ratio	50	0.6	0.1	0.6	0.5-0.6
CSF WBC *	53	324.6	651.8	96.5	25.0 - 312.0
CSF PMN%	52	56.8	28.8	65.0	30.0-80.0
CSF L%	53	44.2	29.6	35.0	20.0-70.5
Serum WBC**	53	12.1	3.8	11.7	9.2-14.0
Serum PMN%	53	74.1	14.7	78.0	67.7-83.8
Serum L%	53	18.5	12.9	14.0	9.8-25.0

*Cells/mm³; ** Values in thousand/L; PMN=polymorphnuclear cells;L=lymphocytes; #25-75P represents the interquatile range.

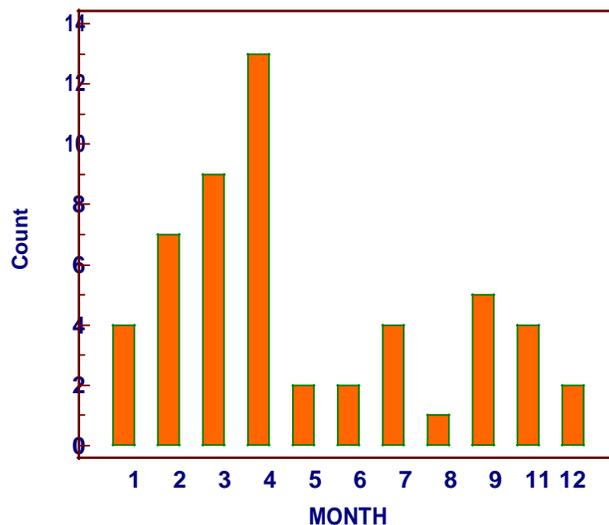


Figure 1: Seasonal variation of pediatric EVM over a 24 month cycle. The Y axis (counts) represents the number of EVM patients seen during each month. Month 1 represents January and month 12 represents December. Note that October (month 10) is not shown as there were no recorded EVM cases.

irrespective of the timing at presentation. This is in variance with most textbook descriptions which describe the typical CSF findings in aseptic meningitis as a pleocytosis between 20 and 1000 white blood cells comprised mainly of lymphocytes (Negrini et al., 2000). This finding is supported by Harrison and Riser who found that 88.9% of their patients maintained a predominance of PMN in the CSF when a repeat LP was performed after 5- 8 h. There are several other studies which support our findings. Lepow et al. (1962) described 407 patients with aseptic meningitis in which 2/3rd had PMN predominance. Chiou et al (1988) reported that 62.5% of 25 infants with Coxsackie' B meningitis had a PMN predominance and

more recently Negrini et al. (2000) reported that 56.5% of 138 cases of aseptic meningitis had a PMN predominance.

Our finding differs from those of earlier studies which suggest a rapid shift from PMN to mononuclear cells (Nye et al., 1983). In addition, a recent study found a mononuclear cell dominance in 51% of patients in the early phase of PCR proven EVM (Shah et al., 2006). In their study, a strong association was not detected between cellular type and the duration of symptoms. The results are at variance with our finding but suggest possibly that the host response may be variable in different geographical settings as a function of the host's immuno-

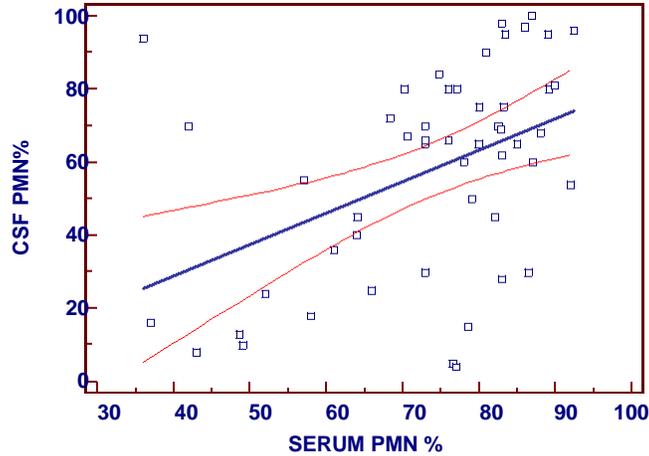


Figure 2: Correlation between CSF and serum PMN% in EVM cases. (Sample size = 51, Pearson's $r = 0.447$, $P = 0.001$, 95% CI = 0.196 to 0.643)

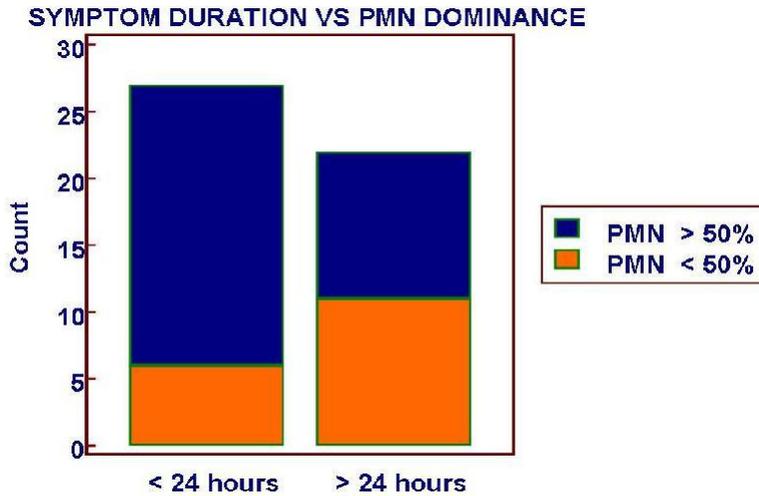


Figure 3. Stacked histogram depicting CSF PMN% in relation to early presentation (<24 h) versus late presentation (>24 h). Count on the Y axis represents the number of patients presenting before 24 hours and the number presenting after 24 h.

genetic response as well as the particular enteroviral serotype implicated. In addition, the possibility of spectrum bias in relation to symptom severity and the selection of patients who have a lumbar puncture may be a reason for the difference compared to their study.

The study limitations include this being a retrospective chart review with rounding of durations of symptoms in some instances. Also we did not perform a diagnostic comparison with bacterial meningitis for the cellular and biochemical characteristics of CSF. Even though length of stay has been considerably decreased in comparison to worldwide standards without the PCR facility, we did not conduct an economic analysis. Our study being retrospective would also introduce an element of spectrum

bias in the selection of patients who actually had a lumbar puncture performed as well as the timing of the lumbar puncture.

Our study is the first to specifically document the serum PMN response and compare it to the CSF PMN response. The correlation found between the two parameters adds credence to the finding of CSF PMN dominance as it makes physiologic sense that the CSF response reflects the serum response, as well as confirming the PMN predominance in the early and late phases of the illness. Other epidemiologic data which were in keeping with prior studies are the male dominance of EVM, which has no obvious explanation. The peak month was in the spring where the temperature is similar to the

summer months of temperate countries, even though we saw cases perennially.

The impact of molecular genetics, by acting as a gold standard and defining a more homogenous and representative sample of enteroviral meningitis cases, has allowed us to more clearly describe physiologic parameters in these patients. PCR diagnosis of EVM also significantly reduces the length of stay and the associated burden of unnecessary antibiotics.

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