An outbreak of echovirus 13 meningitis in central Israel

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SUMMARY

Until recently, echovirus 13 has been a very rare cause of aseptic meningitis. We investigated an outbreak of echovirus 13 in central Israel during the summer of 2000 using a prospective case control study and a retrospective study. Echovirus 13 was isolated from 79 cerebrospinal fluid (CSF) specimens from different medical centres in central Israel. Patients' ages ranged from 10 days to 41 years (95 % < 15 years, M/F ratio 62/38). A total of 128 patients with clinical aseptic meningitis were admitted to the Department of Pediatrics during the outbreak (aged 10 days to 18 years, mean 5·4 years), and 58 CSF samples were processed for viral cultures. Thirty of them did not grow any virus, 26 samples yielded echovirus 13, and 2 samples echovirus 7. The clinical features of patients with echovirus 13 in the CSF were similar to those in whom no virus was isolated or those infected with other enteroviral strains except for higher rate of fever on admission, and prolonged time with fever following the diagnosis in the echovirus 13 patients. CSF cell count varied from 4 to 2333 cells\mm³ with polymorphonuclears (PMN) predominant in > 90 % of our patients. In a case–control study there was no significant difference between patients and matched controls with regard to parameters such as: day care attendance, recreation in summer camp, swimming pools and at the beach, and consumption of tap water. All the patients in our series recovered fully with no neurological abnormalities. The illness caused by echovirus 13 was benign and involved mainly patients younger than 15 year of age. Several features that characterized this outbreak include relatively high WBC in the blood and a prominent CSF PMN response.

INTRODUCTION

Enteroviruses, members of the picornavirus family, are the main cause of aseptic meningitis in the summer. Among the 31 serotypes of echoviruses, types 4, 6, 9 and 30 have been most commonly associated with outbreaks of aseptic meningitis [1–4]. The clinical, laboratory and epidemiological features of aseptic meningitis caused by these agents have been well characterized [1–10]. Viral meningitis associated with echovirus 13 was reported recently in the United Kingdom in 2000 [11] and in the United States in 2001 [12]. We report an outbreak of aseptic meningitis caused by echovirus 13 during the summer of 2000 in central Israel, and describe the clinical and laboratory characteristics of patients admitted to the Department of Pediatrics in Wolfson Medical Center. We also

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conducted a case–control study examining several risk factors for the acquisition of enteroviral aseptic meningitis.

PATIENTS AND METHODS

An increase in number of patients with aseptic meningitis admitted to the paediatric ward in Wolfson Medical Center in Holon, Israel was noticed at the beginning of summer 2000. We reviewed the charts of all patients admitted from 1 July until 1 September 2000.

The diagnostic criteria for aseptic meningitis were as follows:

(1) manifestations of fever or headache or vomiting or restlessness or drowsiness *and*
(2) age appropriate high white blood cell count (WBC) in cerebrospinal fluid (CSF) *and* negative CSF bacteriological culture.

In addition, cases without high CSF cell count who had a positive CSF viral culture, and negative CSF bacteriological culture were also considered as aseptic meningitis.

Viral culture

For virus isolation, three different cell lines were used: human rhabdomyosarcoma cells (RD), buffalo green monkey cell line (BGM), and a local human kidney cell line (HuKi). All three cell lines were sensitive to the recent strain of echovirus 13. The first isolates were identified by performing microneutralization assays, as described by Egberston and Mayo [13], using enterovirus antisera pools [from National Institute of Public Health and the Environment (RIVM), Bilthoven, The Netherlands]. The rest of the isolates were identified by monovalent specific antisera to echovirus 13 (NIH, Bethesda, MD, USA). This method excludes the presence of a second virus in the tissue culture.

Epidemiological analysis

Epidemiological analysis was as follows:

(1) Laboratory and clinical characteristics of patients with echovirus 13 meningitis *vs.* patients with CSF viral culture that was either negative or positive for a different virus.
(2) Laboratory and clinical characteristics of patients < 1 year of age *vs.* ⩾ 1 year.
(3) Case–control study.
(4) Data from Tel Aviv Health District.

Case–control study

A research coordinator specifically trained for conducting this survey interviewed 84 patients or parents of those too young to be interviewed diagnosed with aseptic meningitis and 62 healthy controls, using a standard questionnaire. Controls were unaffected children of similar ages residing in the patients' neighbourhoods. Their names were retrieved from the local Family Health Centres.

Risk factors investigated at the interview included: number of people residing in household, day care attendance, participation in summer camp, time spent in swimming pools and at the beach, eating at restaurants and consumption of tap water.

Statistical analysis

A χ^2 test was performed for comparing the distribution of potential risk factors between cases and controls. Student's *t*-test was used for comparison of continuous variables. Pearson correlation was conducted to find linear relationship between continuous variables.

RESULTS

Patients hospitalized at Wolfson Medical Center

One hundred and twenty-eight patients were admitted to The Department of Pediatrics at The Wolfson Medical Center in Holon in central Israel between 1 July and 1 September 2000 with the diagnosis of aseptic meningitis compared to 75 patients in the same time period 1 year earlier (70 % increase). Their ages ranged from 10 days to 18 years (mean age 5·4 years) with 19 patients younger than 1 year and 10 (8 %) younger than 3 months. All but 3 (98 %) of the patients were less than 15 years. There were 95 males and 33 females. The male/female ratio was significantly different between echovirus 13 patients (ratio = 1·6) and the rest of the patients (ratio = 4·1, $P < 0.0001$).

Laboratory findings

Fifty-eight out of the 128 CSF samples were randomly processed for viral culture. Thirty of them did not grow any virus, 26 grew echovirus 13, and 2 grew echovirus 7. All the bacteriological CSF cultures were negative.

Blood from all 128 patients showed a high WBC count ($> 15\,000/\mu l$) in 25 (20 %) of patients. On CSF analysis ($n = 128$ samples), cells count varied from 4 to

Table 1. *Clinical features in 128 patients with aseptic meningitis*

| | All patients (%) | Age | | P value* |
		≥1 year (n=109) (%)	<1 year (n=19) (%)	
Fever	51	46	85	<0·0001
Rash	11	7	37	<0·0001
Vomiting	81·7	90	29	<0·0001
Diarrhoea	24	28	5	<0·0001
Restlessness	11·6	4	44	<0·0001
Drowsiness	24·7	20	41	<0·0001
Headache		91	NA	
Photophobia		61	NA	
Abdominal pain		24	NA	
Antibiotic treatment before admission	9·4	6	26	<0·0001
Fluid therapy in hospital	75·8	82	42	<0·0001
Antibiotic treatment in hospital	50	42	95	<0·0001

* For comparison between infants <1 year of age and older patients.

2333 cells/μl. Differential CSF cell count revealed polymorphonuclear (PMN) predominance in more than 90% of our patients (mean PMN 79%). The mean protein concentration was 43 mg/dl with values ≥60 mg/dl in 20% of the cases. CSF glucose concentration range from 46 to 99 mg/dl (mean 70·3).

There was a direct correlation between the WBC count in blood and the cell count in CSF ($r = 0·48$, $P < 0·0001$). Significant inverse correlation was found between the number of days until diagnosis and the WBC count in blood ($r = -0·12$, $P < 0·001$) and the percentage of PMN in CSF ($r = -0·25$, $P < 0·007$). However, PMN predominance was also noted even in patients presenting 4 or more days after the onset of symptoms (mean PMN 71%).

Clinical features

Patients presented to the Emergency Room on average 1·7 days after the onset of symptoms (range 0–7 days). Frequent symptoms on admission were: vomiting 105 patients (82%), headache 99 patients (91% of patients >1 year of age), and fever ≥38 °C 65 patients (51% of all patients) (Table 1).

Twelve (9%) children were treated with oral antibiotics before the diagnosis. On physical examination, neck stiffness was present in 91 (71%) of the cases, the Brudzinski sign in 67 (52%) and the Kernig sign in 18 (14%). In the infants, the fontanelle was bulging in 10 patients (53%). Except for the meningeal signs, the rest of the neurological examination was normal. Fifteen patients (12%) had a rash. Fever ≥38 °C was significantly more common on admission in echovirus 13 patients than in the patients with negative viral culture or with echovirus 7 positive culture (77 *vs.* 34·5% respectively, $P = 0·002$). Also, the number of days of fever after the diagnosis was significantly higher in the group of echovirus 13 patients (1·38 *vs.* 0·97 days, $P = 0·03$). However, there was no difference between these groups in the time elapsed from onset of illness until the performance of LP. We did not find additional differences between echovirus 13 patients and the other patients.

Differences between patients younger or older than 1 year

Nineteen (15%) out of 128 patients were younger than 1 year of age. Symptoms such as diarrhoea and vomiting were more frequent in the older group of patients, while fever, rash and drowsiness were more likely to involve the younger group of patients (Table 1). Younger patients were more likely to be treated with antibiotics before admission and to receive antibiotics in hospital.

With regard to laboratory results, younger patients presented with higher CSF cell count, higher protein and lower glucose concentrations in the CSF compared to the older group of patients. There was an inverse correlation between the patients' age and the WBC

Table 2. *Laboratory findings in blood and CSF samples* (n = *128*)

	Mean (range)	Mean in ⩾1 year	Mean in <1 year	P value*
Blood				
WBC (/μl)	11 697 (4800–24 800)	11 343	13 831	NS
Neutrophils (%)	70·4 (7·1–92·7)	73·74	39·03	<0·0001
Lymphocytes (%)	19·8 (4–82)	15·1	47·6	<0·0001
Platelets (/μl)	313 865 (159 000–634 000)	297 238	411 742	<0·0001
Glucose (mg/dl)	114 (78–166)	115·1	111·3	NS
CSF				
Cells (/μl)	199 (4–2333)	148·6	523	0·04
PMN (%)	79 (40–95)	78·4	82·2	NS
Protein (mg/dl)	43 (19–143)	40·9	58	0·026
Glucose (mg/dl)	70·3 (46–99)	71·6	60·7	<0·0001

* For comparison between infants <1 year of age and older patients.

count in the blood ($r = -0·27$, $P < 0·003$) and in the CSF ($r = -0·23$, $P < 0·014$ respectively) (Table 2).

Treatment and re-hospitalizations

Average duration of hospitalization was 2·2 days. Seventy-five per cent of the children received fluids on admission to the hospital. Fifty per cent of the children were treated with antibiotics until the results of the bacteriological cultures came back negative. Younger patients were more likely to be treated with antibiotics but less likely to receive IV fluids.

Fourteen children, all of them older than 1 year of age, were re-hospitalized within 7 days from discharge. Causes of re-hospitalization were vomiting, headache and backache. Five of these children underwent a second lumbar puncture which was negative bacteriologically.

All the patients in our group recovered fully and no neurological abnormalities were detected in follow-up visits at the outpatients' clinic.

Case–control study

The distribution of several risk factors in cases and controls is shown in Table 3. There were no significant differences between cases and controls with regard to the parameters studied.

Tel-Aviv Health District data

In the Central Virology Laboratory in Israel there were no echovirus 13 isolates from the CSF in the 10 years that preceded the outbreak reported here. From the beginning of June 2000 to the end of September 2000, echovirus 13 was isolated from 79 CSF specimens (including our 26 patients with echovirus 13). Sixty-two per cent of the patients with aseptic meningitis reported to the Central Virology Laboratory were male. The patient's age ranged from 10 days to 41 years; 56% were aged 0–4 years, 39% 5–14 years, about 3% were aged 15–19 years and another 3% >20 years. All the 53 patients with echovirus 13 aseptic meningitis who were hospitalized in other medical centres recovered fully (The Ministry of Health, The Tel Aviv Health District, unpublished data).

DISCUSSION

Until recently, echovirus 13 has been rarely if ever described as a significant cause of aseptic meningitis. In the year 2000, laboratories in England and Wales

Table 3. *Odds ratios of parameters in patients as compared to controls in case control study*

Parameter	Rate in cases (%)	Rate in controls (%)	Odds ratio (95% CI)	P value
Participation in camp	46	43·5	1·12 (0·55–2·3)	NS*
Recreation at the beach	28·9	34·4	0·78 (0·36–1·7)	NS
Recreation in swimming pool	72·6	70	1·14 (0·51–2·5)	NS
Eating in restaurants	40	50	0·67 (0·32–1·4)	NS
Day care attendance	8·5	13·7	0·59 (0·17–2·02)	NS
Tap water consumption	43·4	46·5	0·88 (0·41–1·9)	NS

* NS, no statistically significant difference.

reported 67 isolates of echovirus 13 (38 from patients with aseptic meningitis) compared with a total of 19 for the preceding 10 years. Sixty-eight per cent of the isolates associated with aseptic meningitis were from patients over 15 years of age [11].

Similarly, echovirus 13 which was rarely detected in the United States (accounting for only 65 of approx. 45 000 isolates reported to the CDC during 1970–2000) was reported from 13 states in 2001 [12]. As of August 2001, the virus was isolated from 76 specimens from 76 patients of whom 47 (62%) were male and most (96%) were aged <15 years. Thus, the age distribution of patients with echovirus 13 in the present outbreak in Israel was very similar to that reported from the United States, but different from that in England and Wales.

One hundred and twenty-eight patients with aseptic meningitis were admitted to the Department of Pediatrics at The Wolfson Medical Center during the summer of 2000. The clinical features of patients with echovirus 13 in the CSF were similar to those in whom no virus was isolated or those infected with other enteroviral strains. However, a higher rate of fever on admission, and slightly longer period of fever was found in the echovirus 13 patients, even though there was no difference between these groups with regard to the time from onset of illness until the performance of LP.

While the male preponderance of echovirus 13 patients was similar to the figures reported in previous report [12], in the rest of patients this was far greater. This difference suggests that some of these patients had an infection with a different agent. In Israel, the different serotypes of enteroviruses account for the majority of aseptic meningitis cases especially in the summer. Mumps virus, which was an important cause in the past, has been virtually eliminated since the addition of mumps vaccine to the regular immunization programme in 1985.

Frequencies of symptoms and signs in patients with aseptic meningitis were different in patients younger or older than 1 year of age. Diarrhoea and vomiting were more frequent in those over 1 year while fever, rash, drowsiness and restlessness were more common in the younger patients. Older patients were more likely to receive IV fluids probably due to higher rates of vomiting and diarrhoea in this group of patients. Physical findings in our study were similar to those described previously except for neck stiffness which was more frequently present in our study (70% of patients) as compared to 36–62% in others [1, 2, 6]. Our patients had also a higher rate of leucocytosis (18·5% of patients with WBC >15 000/μl in blood) than reported previously [2].

In contrast to a previous study [2], we found an inverse correlation between the patient's age and the WBC in blood and the cell count in the CSF.

The differential CSF cell count revealed PMN predominance in more than 90% of our patients. This finding is in contrast to several studies and to the general belief that enteroviral meningitis is characterized by CSF mononuclear predominance [14–16]. It may be partially explained by the short time that elapsed from onset of illness to lumbar puncture in our patients (1·7 days on average) [17, 18]. However, even though an inverse correlation was found between the number of days until diagnosis and the percentage of PMN in CSF, a PMN predominance was also

noted even in patients presenting 4 or more days after the onset of symptoms. Similar findings were described recently [19].

Our findings emphasize that PMN predominance in the CSF is not a useful tool for discrimination of aseptic from bacterial meningitis.

We investigated in a case–control study, whether the acquisition of aseptic meningitis was associated with a common source of water, food, or day care attendance, as suggested in some reports [20, 21]. However, there were no significant differences between patients and controls with regard to these risk factors.

Half of our patients, including 18 of the 19 patients younger than 1 year, were treated initially with antibiotics until the results of the bacteriological cultures were reported as negative. This underlines the importance of maintaining PCR technique for rapid identification of enteroviral infection in order to avoid the unnecessary usage of antibiotics [22, 23].

The prognosis of echovirus 13 patients in our hospital as well as in other medical centres in central Israel was good. Fourteen patients with aseptic meningitis were re-hospitalized but all patients recovered fully without any neurological sequellae.

In summary, echovirus 13, a rare cause of aseptic meningitis in the past, was an important cause of an outbreak of meningitis. The illness caused by this strain was benign and according to the reports from the Central Virology Laboratory, involved mainly infants and children younger than 15 years of age. Several features that characterized this outbreak include higher than expected of WBC in the blood and high CSF cell count in the younger group of patients and a prominent PMN predominance in the CSF.

REFERENCES

1. Sawyer MH. Enterovirus infections: diagnosis and treatment. Pediatr Infect Dis J 1999; **18**: 1033–40.
2. Gondo K, Kusuhara K, Take H, et al. Echovirus type 9 epidemic in Kagoshima, Southern Japan: seroepidemiology and clinical observation of aseptic meningitis. Pediatric Infect Dis J 1995; **14**: 787–91.
3. Singer JI, Maur PR, Riley JP, Smith PB. Management of central nervous system infections during an epidemic of enteroviral aseptic meningitis. J Pediatr 1980; **96**: 559–63.
4. Muscovite A, Yeti S, Russell A, et al. Echovirus type 4 and 9 in an outbreak of aseptic meningitis in Jerusalem. Scand J Infect Dis 1970; **2**: 87–93.
5. Matsubara T, Matsuoka T, Katayama K, et al. Mononuclear cells and cytokines in cerebrospinal fluid of echovirus 30 meningitis patients. Scand J Infect Dis 2000; **32**: 471–4.
6. Gosbell I, Robinson D, Chant K, et al. Outbreak of echovirus 30 meningitis in Wingecarribee Shire, New South Wales. Commun Dis Intell 2000; **24**: 121–4.
7. Trallero G, Casas I, Tenorioo A, et al. Enteroviruses in Spain: virological and epidemiological studies over 10 years (1988–97). Epidemiol Infect 2000; **124**: 497–506.
8. Mohle-Boetani JC, Matkin C, Pallansch M, et al. Viral meningitis in child care center staff and parents: an outbreak of echovirus 30 infections. Publ Health Rep 1999; **114**: 249–56.
9. Schumacher JD, Chuard C, Renevey F, et al. Outbreak of echovirus 30 meningitis in Switzerland. Scand J Infect Dis 1999; **31**: 539–42.
10. Ashwell MJ, Smith DW, Phillips PA, et al. Viral meningitis due to echovirus types 6 and 9: epidemiological data from Western Australia. Epidemiol Infect 1996; **117**: 507–12.
11. Anonymous. Viral meningitis associated with increase in echovirus type 13. CDR 2000; **10**: 277–80.
12. Anonymous. Echovirus type 13 – United States, 2001, Centers for Disease Control. MMWR 2001; **50**: 777–80.
13. Egbertson SH, Mayo DR. A microneutralization test for the identification of enterovirus isolates. J Virol Methods 1986; **14**: 305–7.
14. Greenlee JE. Approach to diagnosis of meningitis. Infect Dis Clin North Am 1990; **4**: 583–98.
15. Nye F. The value of initial laboratory investigations in the management of meningitis. J Infect 1983; **7**: 31–8.
16. Singer JI, Maur PR, Riley JP, et al. Management of central nervous system infections during an epidemic of enteroviral aseptic meningitis. J Pediatr 1980; **96**: 559–63.
17. Feigin RD, Shackelford PG. Value of repeat lumbar puncture in the differential diagnosis of meningitis. N Engl J Med 1973; **289**: 571–4.
18. Amir J, Harel L, Frydman M, et al. Shift of cerebrospinal polymorphonuclear cell percentage in the early stage of aseptic meningitis. J Pediatr 1991; **119**: 938–41.
19. Negrini B, Kelleher KJ, Wald ER. Cerebrospinal fluid findings in aseptic versus bacterial meningitis. Pediatrics 2000; **105**: 316–9.
20. Melnick JI, Gerba CP. The ecology of enteroviruses in natural waters. CRC Crit Rev Environ Control 1980; **10**: 65–93.
21. Keswick BH, Gerba CP, Goyal SM. Occurrence of enterovirus in community swimming pools. Am J Publ Health 1981; **71**: 1026–30.
22. Ramers C, Billman G, Hartin M, et al. Impact of diagnostic cerebrospinal fluid enterovirus polymerase chain reaction test on patient management. JAMA 2000; **283**: 2680–5.
23. Robinson CC, Willis M, Meagher A, et al. Impact of rapid polymerase chain reaction results on management of pediatric patients with enteroviral meningitis. Pediatr Infect Dis J 2002; **21**: 283–6.