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Short reports

Detection of *Clostridium difficile* enterotoxin in neonates by latex agglutination

H USHIJIMA, T SHINOZAKI, AND R FUJII

Department of Paediatrics, Teikyo University School of Medicine, Tokyo, Japan

SUMMARY *Clostridium difficile* enterotoxin (D-1) was detected in 13 symptomatic and nine asymptomatic neonates in the neonatal intensive care unit by latex agglutination test but was not found in 18 healthy neonates in two other newborn nurseries. Environmental contamination in the intensive care unit may have been the cause. An association between the presence of enterotoxin and clinical symptoms is discussed.

Clostridium difficile toxin is considered to be the cause of pseudomembranous colitis and necrotising enterocolitis.[1] Its cytotoxin was detected in faeces of symptomatic and asymptomatic infants in a neonatal intensive care unit.[2] Cytotoxic assay in cell cultures with neutralisation by *C difficile* antitoxin has been a useful test for detecting *C difficile* cytotoxin. *C difficile* enterotoxin has shown similar biologic activity in the rabbit ilial loop test to that of *Escherichia coli* enterotoxin. Latex agglutination test for *C difficile* enterotoxin, first developed by Kohno (personal communication), is a simple, rapid, and sensitive test.

Two small outbreaks of diarrhoea have occurred in our neonatal intensive care unit in the past year. Rotavirus was detected in the first outbreak in June 1983 by polyacrylamide gel analysis of genome ribonucleic acid and *C difficile* was isolated by bacterial culture in the second outbreak during January and February 1984. The present study, undertaken between October 1983 and February 1984, was designed to detect *C difficile* enterotoxin by latex agglutination test in neonates in the intensive care unit. Neonates in two newborn nurseries were also examined as controls.

Materials and methods

All infants admitted to the intensive care unit during the study period were studied. Two hundred and thirty stool specimens from 59 patients in the unit were collected once or twice weekly in polystylene vials. Sixty stool specimens from 18 healthy neonates in two newborn nurseries were also collected. These specimens were stored at −20°C until tested.

Anti- *C difficile* enterotoxin (D-1) antisera were made in rabbits. The IgG fraction of antisera was purified and adsorbed to polystylene beads. Latex agglutination test kits were kindly provided by Dr H Kohno, Mitsubishi-kasei Research Centre and Dr M Ueno, Gifu University, Japan. A value of over 500 ng/ml of D-1 toxin was considered to be a positive result.

Approximately 0·2 g of faeces and 0·2 ml of dilution buffer (0·2 M Tris-Hcl, 0·9% Nacl, 0·1% bovine serum albumin) in microcentrifugation tubes were mixed and centrifuged at 6000 *g* for 10 minutes. Fifty µl of supernatant was mixed with 10 µl of anti- D-1 antisera coated latex for three minutes on black glass plate and agglutinations were observed under the transmitted light. Pretreatment of supernatant with 2 µl of anti- D-1 antisera for one minute was undertaken to check for any false positive reaction.

Results

C difficile D-1 enterotoxin was detected in 37% (22 of 59) of neonates in the intensive care unit more than once in the five-month period, and 0% (0 of 18) in two newborn nurseries. Twenty per cent (46 of 230) of total specimens tested were positive in the intensive care unit neonates. Stools from more than half the neonates in the unit were positive at the end of January 1984, and toxin was nearly always found in some faeces of a few neonates during the study period. (Fig. 1). Thirteen of 22 toxin positive neonates had diarrhoea while the other nine were asymptomatic. Only six of 22 toxin positive neonates received antibiotics before or during the time their faeces showed a positive result; the remaining 16 had not received antibiotics. These results indicated a close association between the presence of toxin and diarrhoea, although there were some cases

Month (1983–1984)	Oct			Nov			Dec			Jan			Feb				
Days	1–10	11–20	21–31	1–10	11–21	21–30	1–10	11–20	21–31	1–10	11–20	21–31	1–10	11–20	21–29	Total	
No positive samples	2	4	0	1	5	3	1	2	0	1	1	19	4	2	1	46[a]	
No samples tested	26	17	8	16	21	12	10	12	7	11	6	45	21	13	15	230[a]	
% positive	8	23	0	6	23	25	10	17	0	9	17	42	33	15	20	20	
No positive neonates	2	4	0	1	3	3	1	2	0	1	1	10	4	3	1	36[b]	22[c]
No neonates tested	19	18	22	20	18	19	17	16	12	14	11	18	11	11	5	231[b]	59[c]
% positive	10	22	0	5	17	16	6	13	0	7	9	56	36	27	20	16	37

Fig. 1 *Occurrence of Clostridium difficile toxin in newborns in an intensive care unit during a five month period.*

(a) Number of positive samples or samples tested in each 10 day period. (b) Number of neonates positive for *C difficile* or neonates tested in each 10 day period. (c) Total number of neonates positive for *C difficile* or neonates tested during study.

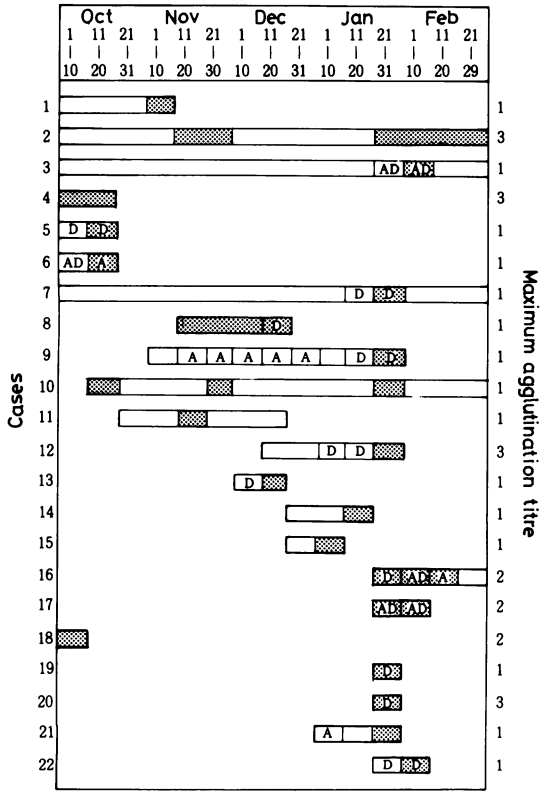

Fig. 2 *Relation between Clostridium difficile toxin, diarrhoea, and antibiotic treatment.*

Twenty two cases were *C difficile* toxin positive during the study. Horizontal bars indicate the duration of study in each case with the shaded areas indicating excretion of *C difficile* toxin.

A=antibiotic treatment. D: diarrhoea.

Agglutination titres: 1 represents 1000 to 500 ng/ml, 2 represents 1000 to 2000 ng/ml, 3 represents over 2000 ng/ml.

where the occurrence of diarrhoea was not related to this. The toxin titre was positive in three of four neonates with or without diarrhoea and prolonged excretion of toxin (Fig. 2). The finding of toxin in neonatal stools was not related to age.

Discussion

C difficile produces two toxins, enterotoxin and cytotoxin. It has been suggested that toxin (D-1), which was used in our study, and toxin A reported by Libby *et al*[3] are identical enterotoxins as are toxin (D-2) and toxin B. There are many reports describing *C difficile*, its cytotoxin, and clinical manifestations in adults and the recovery of the organisms and cytotoxin from stools of neonates have been shown in some.[1 2] Only Libby *et al*[3] have reported the relation between *C difficile* toxins A and B in infants.

It is possible that the absence of clinical manifestations in some neonates whose stools show high cytotoxic titres may be explained by low titres of enterotoxin or by a non-enterotoxin producing strain.[1] Our study shows that *C difficile* enterotoxin can be detected in stools of a large number of symptomatic and asymptomatic newborns in the intensive care unit. This means that the presence of enterotoxin cannot explain why some asymptomatic neonates have *C difficile* cytotoxin in stools. Libby *et al*[3] suggested that certain infants are protected from the effects of toxins because, for example, they lack toxin receptors or the means for further processing of the toxin.

Toxin was nearly always found in the stools of some infants in the intensive care unit regardless of whether antibiotics were used. Toxin was not detected in two newborn nurseries one of which was located on a different floor of the same hospital and

the other in a different hospital. Breast feeding and vaginal delivery were not associated with high frequencies of toxin detection in the intensive care unit (data not shown). These findings suggest that nosocomial factors influence the existence of *C difficile* and the outbreak of diarrhoea associated with *C difficile* in the intensive care unit.

Latex agglutination test is a simple and quick method compared with enzyme linked immunosorbent assay.[4] *C difficile* is recovered from all enterotoxin positive stools and is not detected in enterotoxin negative stools.[4] The method is sensitive to 15 ng/ml of enterotoxin, and can be useful for detecting diarrhoea, especially antibiotic-associated colitis for children and for adults, even though *C difficile* is sometimes found in asymptomatic neonates.

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Correspondence to Dr Hiroshi Ushijima, Department of Paediatrics, Teikyo University School of Medicine, Kaga 2–11–1, Habashi-ku, Tokyo 173, Japan.

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Medical management of bilateral renal malakoplakia

E B CHARNEY, C L WITZLEBEN, S D DOUGLAS, N KAMANI, AND M A KALICHMAN

Departments of Pediatrics and Pathology, University of Pennsylvania School of Medicine, and Department of Child Development and Rehabilitation, Pathology, Allergy and Immunology, Children's Hospital of Philadelphia

SUMMARY Renal malakoplakia is reported in an 11 year old girl with myelomeningocele and associated neuropathic bladder. She is the first reported child to have survived bilateral renal malakoplakia with full recovery of renal function after medical management.

Malakoplakia is an unusual chronic inflammatory response primarily occurring in debilitated adults, and characterised by aggregates of macrophages containing concentric calcospherites or Michaelis-Gutmann bodies. An immunologic defect of ineffective macrophage phagocytic ability is believed to contribute to its pathogenesis. Although frequently affecting the collecting system of the urinary tract, it only rarely involves renal parenchyma. Earlier published reports[1 2] state that unilateral renal involvement requires nephrectomy and bilateral involvement is invariably fatal. We report a child with bilateral renal malakoplakia in whom conservative medical management resulted in survival and normal renal function.

Case report

An 11 year old girl was admitted to this hospital with a five day history of fever and abdominal pain. Past medical history included myelomeningocele repair and ventriculoatrial shunt for hydrocephalus at birth with a shunt revision at age 9 years and insertion of a Brantley-Scott prosthetic urethral sphincter for urinary incontinence at age 8 years. According to parental reports, the child had not complied in deactivating the sphincter regularly during the previous few months. Two years before hospital admission her intravenous pyelogram had been normal, blood urea nitrogen (BUN) was 3·9 mmol/l (10·9 mg/100 ml) and serum creatinine was 44·2 μmol/l (0·5 mg/100 ml).

Physical examination on admission showed a toxic appearing child with temperature of 38·2°C; heart rate 136/minute; respiratory rate 24/minute; blood pressure 132/80 mm Hg and bilateral flank tenderness with diffuse abdominal pain. Laboratory evaluation on admission included the following values: haemoglobin 10·1 g/dl; haematocrit 28%, white cell count $5{\cdot}7\times10^9$/l with 77% segmented neutrophils; 1% eosinophils; 19% lymphocytes and 3% monocytes; and platelets 98×10^9/l; erythrocyte sedimentation rate 63 mm in the first hour. Serum sodium was 137 mmol/l; potassium 2·8 mmol/l; chloride 103 mmol/l; bicarbonate 16 mmol/l; BUN 23 mmol/l (61·3 mg/100 ml); and serum creatinine 256 μmol/l (2–9 mg/100 ml). Urine obtained by bladder catheterisation (800 cc) had a specific gravity of 1·010, trace protein, and numerous white