1977

ENTEROTOXIGENIC ESCHERICHIA COLI DISEASE IN BANGLADESH:

CLINICAL, THERAPEUTIC AND LABORATORY ASPECTS

Michael H. Merson, R. Bradley Sack, Sirajul Islam, Golam Saklayen, Nural Huda, A.K.M. Golam Kibriya, Abdullah-al Mahamood, Qazi Shafi Adamed, Anwarul Qudur, Mujibur Rahaman

Extensive studies of large members of patients with diarrheal disease due to enterotoxigenic E. coli (ETEC) have not previously been done. We have attempted to prospectively study, in a holistic fashion, the cholera-like clinical illness caused by these organisms, and to determine whether antibiotic therapy is useful in the clinical management of these patients.

In the fall of 1976, we studied 176 consecutive adult males (> 10 yrs. of age) admitted to the Cholera Research Hospital in Dacca, Bangladesh with acute cholera-like diarrhea. Patients were selected who had not received prior antibacterial therapy and were screened on admission by dark field examination of direct stool specimens to eliminate these patients with cholera. The etiology of the patients' diarrheal diseases are shown in Table I. In approximately 85% of patients, an etiologic agent could be identified, the most common being ETEC (55%). Mixed infections were primarily with E. coli, Shigella, and non-cholera vibrios.

The clinical and therapeutic aspects of 45 patients with disease due to E. coli producing both heat labile enterotoxin (LT) and heat stable enterotoxin (ST) and 18 patients with disease due only to ST- producing E. coli were studied in detail. (There were too few patients (3) harboring LT-only producing E. coli to give comparable data.) The clinical features on admission of these patients are given in Table II. No significant differences were found, although patients with LT-ST disease tended to be more severely dehydrated on admission, with higher serum specific gravities, and lower plasma bicarbonates. Illness due to LT-ST organisms was significantly more severe, however, as measured by a longer duration of illness after reaching the hospital and excretion of larger total stool volumes. (Table III).

To study the effect of antibiotic therapy on ETEC disease, patients were randomized into tetracycline (500 mg every 6 hours for 3 days (250 mg doses for persons under 30 kg in weight) or placebo treatment groups in a double blind fashion. Fluid therapy of the patients was identical. Patients with mixed infections or with tetracycline-resistant organisms were excluded from the analysis of the results.

The results in patients with LT-ST diarrheal disease are given in Table IV and Fig. 1. The clinical and biochemical parameters of the patients in the two groups on admission (data not shown) were identical. Tetracycline significantly shortened the duration of excretion of ETEC ($p < 0.001$), and uniformly terminated the clinical illness within 28 hours after hospitalization ($p = 0.025$).

JH 1976-77

The results in patients with ST-only disease are given in Table V. The clinical illness, only 17 hours after admission in the placebo group, was not shortened by antibiotic therapy. ETEC excretion, however, was significantly shortened ($p < 0.05$).

Antibiotic sensitivity testing was done on all ETEC isolates as well as a sampling of non-ETEC organisms. LT-ST organisms were more sensitive to antibiotics than either ST-only organisms ($p = 0.06$) or non-ETEC ($p = 0.02$).

Antitoxin titres to LT were measured in paired sera from all individuals by neutralization in adrenal cell tissue culture. These results, shown in Table VI, indicated that most patients (62%) harboring LT-producing E. coli had significant rise in titre, whereas no patient harboring ST-only E. coli developed a significant antibody response.

Using the Y-1 adrenal and Chinese Hamster ovary cell assays to detect LT and the infant mouse assay to detect ST, we found that testing pools of 10 ETEC isolates was less sensitive than testing individual isolates when less than 7 of 10 isolates in the pool were enterotoxigenic. However, in 95% of these patients, 9 of 10 isolates tested per patient were found positive, so that testing of a single colony would have been adequate to make a laboratory diagnosis.

The serotypes of the ETEC were determined by Dr. Ørskov of the WHO Reference Laboratory in Copenhagen. The majority (57%) of LT-ST E. coli fell into 5 major "O" groups (O6, O8, O15, O25, O78, O115) whereas, the ST-only E. coli were scattered into approximately 15 "O" groups, with no predominance of any.

Additional work done as part of this study includes: 1) studies on the detection of LT antigen in direct stool samples by the adrenal cell assay, and radio-immune and ELISA assays, in collaboration with Dr. Kapikian's lab at NIH, and 2) studies on the detection of ST in direct stool samples by the infant mouse test. These data are not yet available for presentation, but the results can be summarized as follows: 1) LT antigen can be detected in stool samples directly about 70% of the time; and the 3 assays correlate well with each other, 2) ST is detected less often in stool directly, only about 35% of the time.

This study demonstrates that in Bangladesh 1) ETEC are a common cause of severe cholera-like diarrhea, 2) that the laboratory diagnosis of ETEC disease can be easily made, and 3) that there are clinical differences between patients depending upon whether the ETEC produce ST and LT or ST-only, 4) that LT-ST disease warrants therapy with tetracycline.

TABLE I

Frequency of organisms from 176 study cases

Organism	Isolated: Single pathogen No.	Single pathogen %	Total infections No.	Total infections %
Enterotoxigenic *E. coli*	95	54	109	62
V. cholerae	12	7	14	8
V. Parahaemolyticus	6	3	6	3
NAG (Non-cholera vibrio)	5	3	14	8
Shigellae	4	2	16	9
Invasive *E. coli*	1	1	1	1
Rotavirus	1	1	9	5
Salmonellae			1	1

TABLE II

Comparative features of LT-ST and ST enterotoxigenic *E. coli* diseases

	LT - ST (N = 31)	ST (N = 16)
Age (years)	33	24 (p = .07)
Discharge weight (kg)	41.6	38.5
Admission systolic BP (mmHg)	76	79
Weight change (%)	7.5	6.3 (p = 0.4)
Clinical severity (cases)		
Moderate	10	9
Moderate - severe	13	6 (p < 0.2)
Severe	8	1
Admission spec. gravity	1.0334	1.0327 (p = .09)
Admission serum bicarbonate (mEq/L)	16.0	17.8 (P < .1)

TABLE III

Comparative features of LT-ST enterotoxigenic E. coli diseases

	LT - ST (N = 31)	ST (N = 16)	
Pre-admission diarrhea (hrs.)	11.9	11.7	
Post admission diarrhea (hrs.)	20.7	10.8	$p < .05$
Total illness (hrs.)	32.6	22.5	$p < .05$
Stool volume (cc/kg)	35	11	$p = .03$
I.V. volume (cc/kg)	111	81	$p < .05$
Stool excretion (days)	2.4	2.1	

TABLE IV

Effect of tetracycline on LT-ST enterotoxigenic *E. coli* disease

	Placebo (N = 25)	Tetracycline (N = 20)	
Pre-admission diarrhea (hrs.)	11.8	11.7	
Post-admission diarrhea (hrs.)	25.0	18.8	($p < 0.2$)
Stool volume (cc/kg)	43	28	($p = 0.34$)
I.V. volume (cc/kg)	120	105	($p < 0.3$)
Stool excretion (days)	2.2	0.6	($p < .001$)

TABLE V

Effect of tetracycline on ST enterotoxigenic E. coli disease

	Placebo (N = 10)	Tetracycline (N = 8)
Pre-admission diarrhea (hrs.)	10.5	8.0
Post-admission diarrhea (hrs.)	17.2	15.5
Stool volume (cc/kg)	17	24
I.V. volume (cc/kg)	85	86
Stool excretion (days)	1.8	0.4 p = .01

TABLE VI

Frequency of four-fold or greater titer rise to *E. coli* heat-labile (LT) enterotoxin by etiology

Etiology	Paired sera	Four-fold or greater titer rise	
	(No.)	(No.)	(%)
Enterotoxigenic *E. coli*			
LT-ST	38	23	61
ST	22	0	0
LT	4	3	75
NAG (non-cholera vibrio)	9	1	11
Shigella	6	0	0
Rotavirus	1	0	0
Unknown	22	0	0

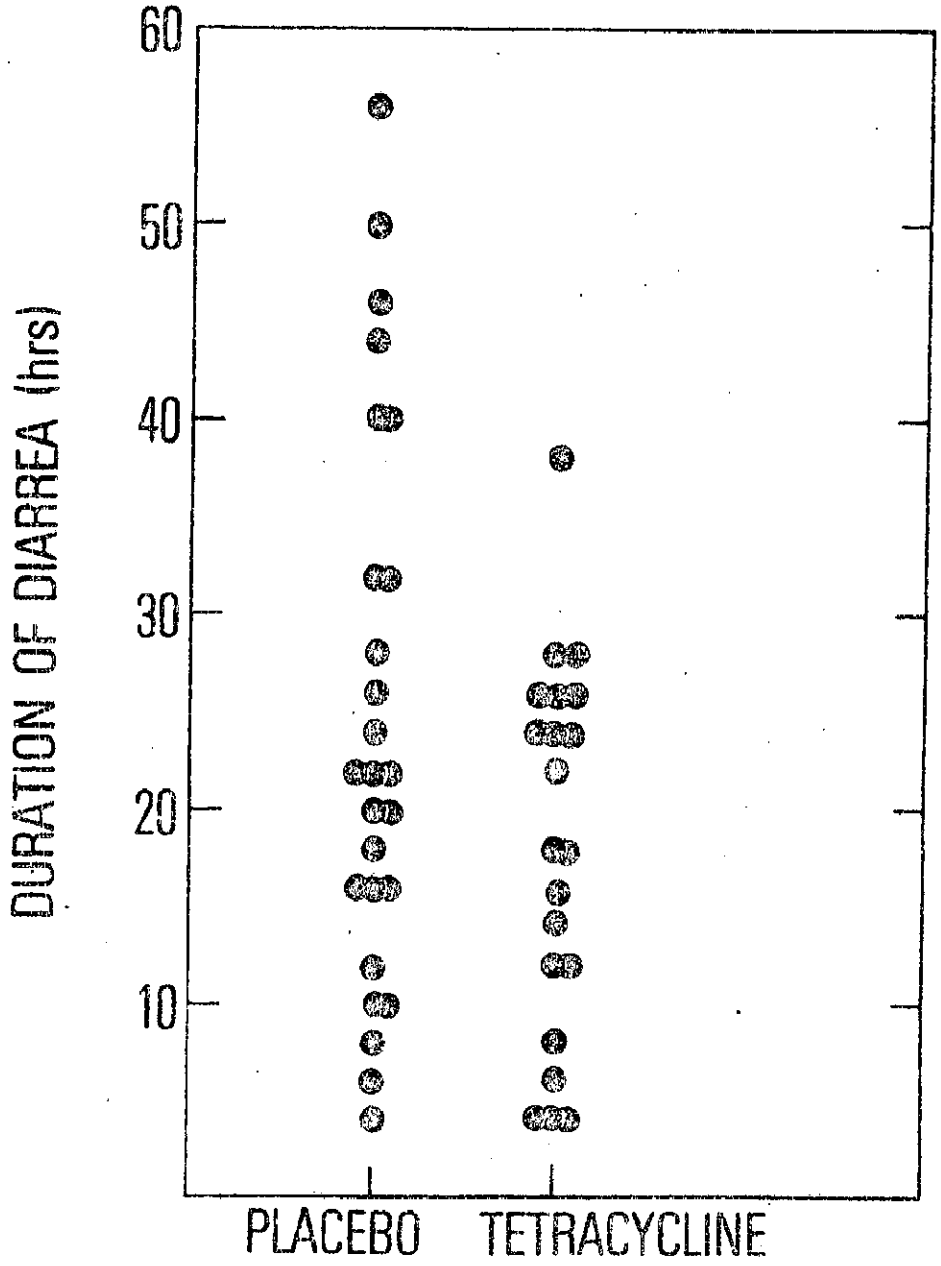

Fig. 1. Duration of diarrhea following admission in patients with LT/ST producing E. coli according to therapeutic groups. Each dot represents one patient. The difference between the groups is