THE JOURNAL OF INFECTIOUS DISEASES • VOL. 151, NO. 6 • JUNE 1985
 0022-18W85/5I06-OO20SO1.O0

Diarrhea Associated with Typhoid Fever

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To study the pathogenesis of diarrhea occurring with typhoid fever, we selected 42 patients with diarrhea and blood cultures positive for *Salmonella typhi* or *Salmonella paratyphi* A, but without diarrhea! copathogens, for measurement of stool output and examination of fecal composiiion. The mean duration of fever before hospitalization was 9.5 days, and the mean duration of diarrhea was 5.8 days. All patients passed liquid stool on their first day in the hospital, ranging in volume from 4 to 172 ml/kg with a mean of 45 ml/kg. Red blood cells were in the stools of 57% of the patients. All patients had fecal leukocytes with a mean of 4,950 leukocytes/mm^3, predominantly polymorphonuclear leukocytes. In the stools, the mean protein concentration was 9.3 g/liter; the mean pH was 6.1, and the mean concentration of electrolytes was as follows: sodium, 47 mEq/liter; potassium, 48 mEq/liter; and chloride, 43 mEq/liter. The mean total COj was 24 mmol/liter. During treatment with chloramphenicol, this group of patients showed daily improvement with a drop in both fever and stool output. The results indicate that patients with diarrhea during typhoid fever have a wide range of rates of purging, and the diarrhea is characterized by liquid stool containing large quantities of leukocytes and protein and is resolved by treatment with chloramphenicol.

Clinical descriptions of typhoid fever emphasize the cardinal features of prolonged fever; abdominal pain, distention, or tenderness; splenomegaly; hepatomegaly; and rose spots. Diarrhea is usually relegated in importance to a common finding that is not regularly present and not diagnostically useful. On the other hand, diarrhea has been reported recently in greater numbers of cases [1]. The reported frequency of diarrhea in patients with typhoid fever was varied by geography from <33% in five reports from India [2-6] to between 33% and 50% from African countries [7-12], Jordan [13], and the United States [14] and to >50% in Chile [15], Ethiopia [16], Nigeria [17], Sweden [18], and the United States [19].

Received for publication August 17, 1984, and in revised form December 5, 1984.

Informed consent was obtained from patients or their parents or guardians. Guidelines for human experimentation of the International Centre for Diarrhoeal Disease Research, Bangladesh were followed in the conduct of the clinical research.

We (hank Abul Alim, J. P. Butzler, H. Conguan, and Drs. Asma Khanam and M. Rahman for technical assistance and Mahbooba Shamsuddin for typing the manuscript.

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These differences in the prevalence of diarrhea in typhoid fever may not be absolute; differences in detection methods and definitions of diarrhea used by the various authors may account for the variation. The frequency is reportedly higher in young children than in older children and adults [4,13], In 1946, Stuart and Pullen [14] described the diarrheal stool of patients with typhoid fever as foul-smelling with a consistency of pea soup and occurring as often as 20 times a day, sometimes with severe and protracted occurrences.

In 1983 Stoll et al. [20] reported in a retrospective study of 62 patients with typhoid fever and diarrhea in Bangladesh that most of the patients showed watery diarrhea with mucus that revealed >10 white blood cells per high-power field (hpf) and had an alkaline pH. The present study planned prospectively to obtain complete quantitative measures of the rates of purging and the fecal composition in patients with typhoid fever and to observe the course of diarrhea during treatment with chloramphenicol.

Patients and Methods

Patients. The International Centre for Diarrhoeal Disease Research, Bangladesh operates a hospital and outpatient clinic in Dhaka for the treatment of patients with diarrhea. All male and female patients, six months to 60 years of age, who were

reportedly from three to 30 times per day (mean$_f$ 10.2 ± 1.1 times). Stools were described as watery in 41 (98%) of the cases, and large amounts of blood had been noted in the stools by only three (7%) of the patients; one patient described the stools as soft. The dehydration detected in these patients by physical examination was mild or absent in all but nine patients, who showed moderate dehydration. The highest value of serum-specific gravity obtained was 1.028, a result confirming that dehydration was never more than moderately severe in these diarrheal cases. The means of serum electrolytes were as follows: sodium, 130 mEq/liter; potassium, 3.5 mEq/liter; chloride, 95 mEq/liter; and total CO_2, 21 mmol/liter.

Characteristics of diarrhea. The volume of stool passed during the first 24 hr in the hospital varied from 4 to 172 ml/kg (mean, 45.2; table I). Blood or flecks of blood were visible in the stools of 7% of the patients, and the Guaiac test was positive for blood in 29% of the patients. Microscopic examination of stools revealed red blood cells in 57% of the patients. Fecal leukocytes were present in all cases. Counting white blood cells in the hemacytometer revealed a range from 400 to 16,500 cells/mm' (mean, 4,950 ± 590 cells/mm^3). Differential white blood cell counts of fecal leukocytes revealed a mean composition of 70% PMNLs and 30% mononuclear cells. The protein concentration was 9.3 ± I.3g/literwithameanpHof6.1. The stool electrolyte values varied over wide ranges.

Hospital courses and outcomes. All 42 patients were treated with chloramphenicol and iv fluids and were allowed to eat a regular hospital diet. Three patients died. Intestinal perforations were detected in three patients by the radiographic demonstration of free air under the diaphragms; two were operated on for repair of the perforations and for peritoneal drainage, and both survived. In the 37 patients who survived and did not experience intestinal perforation, the responses of fever and diarrhea to treatment were noted daily (figure 1). There were consistent downward trends in both fever and the rates of diarrheal purging after the start of therapy.

Tests of toxigenicity and invasiveness of S. typhi. *S. typhi* strains from 39 patients were examined for heat-labile toxin and heat-stable toxin and for positivity in the Sereny test. All strains gave negative results, which suggests that t he diarrheal mechanisms in diarrhea associated with typhoid were distinct from those of enterotoxigenic *E. coli* and *Shigella* species.

Table 1. Clinical Features and fecal composition of 42 patients with blood-culture positive typhoid fever and diarrhea.

Clinical features	Mean ± SE or percentage of patients with feature	Range
Prehospital duration of:		
Fever (days)	9.5 ± 0.9	2-30
Diarrhea (days)	5.8 ± 0.5	1-20
No. of stools passed during 24 hr before admission	10.2 ±1.1	3-30
Stool characterization:		
Watery	98%	
Soft	2%	
Bloody	7%	
Rale of purging on admission (ml/kg body weight per day)	45.2 ± 5.3	4-172
Positive Guaiac icsi	29%	
Fecal rbc per hpf:		
0	40%	
1-9	52%	
&10	5%	
Feca! wbc per hpf:		
0-19	74%	
20-49	21%	
£50	5%	
Fecal wbc per mm^J	4.950 ± 590	400-16.500
Fecal PMNLs (%)	70 ± 3	30-100
Feca! mononuclear cells (<7o)	30 ± 3	0-70
Protein (g/liler)	9.3 ± 1.3	3-26
pH	6.1 ± 0.2	4.5-7.8
Slool electrolytes (mEq/liter):		
Sodium	47 ± 5	3-118
Potassium	48 ± 5	14-137
Chloride	43 ± 3	8-84
Total CO; (mmol/liter)	24 ± 3	6-92

NOTE, Rbc, red blood cells; wbc. while blood cells; and hpf, high-power field.

Discussion

All of the 42 patients described in this study who had enteric fever that was blood-culture positive had diarrhea. Although these patients were selected for admission to a hospital on the basis of their diarrhea, diarrhea has been reported to occur in over half of all patients with typhoid fever as reported in several studies in other countries, a finding indicating that diarrhea is a common and important symptom of this disease [15-19]. This occurrence of diarrhea in patients with typhoid fever who naturally acquired the disease contrasts with the disease course

consulting the outpatient clinic with a history of fever and diarrhea for four or more days and who had not received chloramphenicol or cotrimoxazole since the start of their illness were eligible for the study. Diarrhea was defined as three or more bowel movements of loose or liquid stools per 24 hr. Initially, fifty[1] patients with a blood culture positive for *Salmonella typhi* OT *Salmonella paratyphi* A were included, but eight were later excluded because they were infected with diarrheal copathogens. Five patients had *Entamoeba histolytica;* two had *Ciardia lamblia;* two had enterotoxigenic *Escherichia coli* that produced heat-labile toxin (LT) or heat-stable toxin (ST); and two had *Shigella* infections. Stool cultures were negative for *Campylobacter* species in all cases. Histories were taken and physical examinations performed. Rectal temperatures, pulse rates, and blood pressures were recorded. Stool volumes were measured every 8 hr by weighing buckets into which patients were instructed to defecate.

Bacteriology. A single blood specimen of 5 ml and a rectal swab were cultured by using standard methods. Isolated strains of *S. typhi* were sent to the Enteric Reference Laboratory (Colindale, England) where Vi-phage-typing was performed (courtesy of Dr. Rowe). The same strains were tested for antimicrobial susceptibility by the method of Bauer et al. [21] and by microdilution for MICs.

Blood and stool examinations. Serum-specific gravity was measured by refractometry, and scrum electrolytes were determined for patients upon admission to the hospital. Stool microscopic examination was performed by passing a wire loop through a random section of stool, making a wet mount preparation, and estimating the number of leukocytes and red blood cells per hpf. Stool samples were examined for the presence of occult blood with the guaiac test. The number of fecal white blood cells per mm^3 was determined for patients upon admission by diluting 1 ml of stool with 2 ml of 0.9% NaCl. The diluted stool was vortexed for 2 min and allowed to stand for 3 min. The upper layer was pipetted and diluted i:iO in 0.1 *N* HC1. The leukocytes were counted in a hemocytometer. Supernatants of stool were obtained for electrolytes and protein measurements after centrifugation at 2,000 *g* for 1 hr. Protein was measured by the biuret method [22], and pH was determined on a Corning pH meter (model 7; Corning Scientific Instruments, NJ); sodium and potassium were measured by flame photometry (IL no. 443). Chloride was measured by a modified Zall colorimetric procedure [23]. CO_2 was measured directly by the same TCO_2 electrode methodology as is used in IL blood gas systems (JL-446) [24].

Treatment. Patients were rehydrated with iv solution containing sodium (133 mEq/liter), potassium (13 mEq/liter), chloride (98 mEq/liter), and acetate (48 mmol/liter). No oral rehydration solution was used. Twenty-nine patients were treated with chloramphenicol orally (Parke-Davis, Dhaka, Bangladesh) and 13 patients were initially treated iv with chloramphenicol sodium succinate (Rachelle Laboratories, Long Beach, Calif) given every 6 hr for a total daily dosage of 60 mg/kg. After defervescence the dosage of chloramphenicot was reduced to 40 mg/kg per day given in four equal doses.

Tests of toxigenicity and invasiveness. S. typhi isolates from 39 patients with different rates of purging vvere tested for invasiveness by the Sereny test. Twenty microliters (5×10^8 cells) of bacterial suspension (from acute disease) in PBS was instilled into the conjunctival sac of guinea pig. The development and severity of lesions of the eye were monitored over a period of seven days from the day of challenge [25]. These strains were tested also for the production of heat-labile toxin by the Chinese hamster ovary cell assay and of heat-stable toxin by the infant mouse assay [26].

Results

Bacterial strains. S. typhi was isolated from blood cultures of 40 patients and from stool cultures of 23 patients. *S. paratyphi* A was isolated from blood cultures of two patients. The distribution of Vi-phage types in *S. typhi* strains tested from 31 patients was as follows: four each of untypable Vi-strain 1 and Vi-negative; three each of Ml, T, 46_f and 53; two each of degraded Vi-strain 4 and E14; and one each of A, F3, F4, Jl, Kl, 40, and 60. Antimicrobial susceptibility testing showed that all 42 isolates were susceptible to ampicillin, chloramphenicol, gentamicin, kanamycin, and trimethoprim-sulfamethoxazole.

Patients. The patients ranged in age from two to 36 years with a median age of 16 years. Males predominated with a male-to-female ratio of 24:18. The medical histories revealed that these patients complained of fever for two to 30 days (mean ± SE, 9.5 ± 0.9 days) before arriving at the hospital. AH patients complained of diarrhea for one to 20 days (mean, 5.8 ± 0.5 days) before admission. On the day before admission, the rate of stool purging was

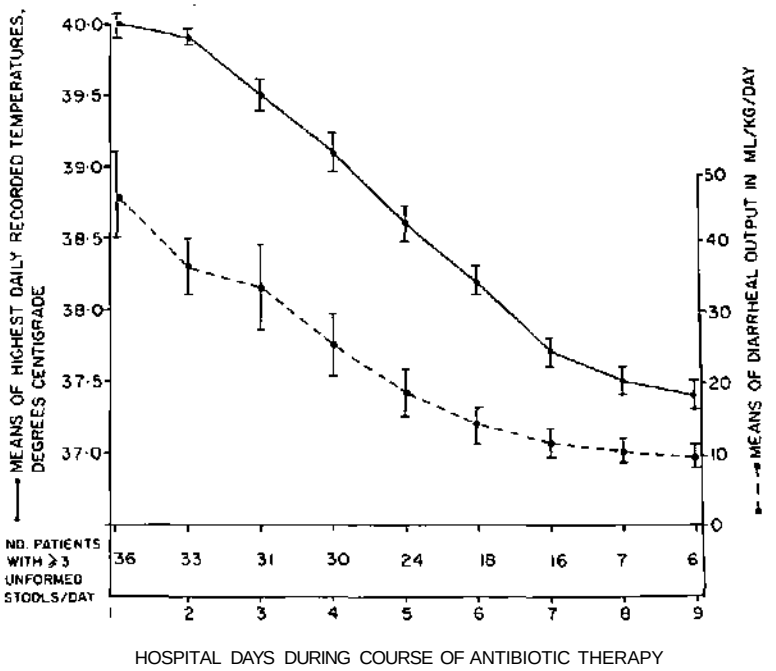

Figure 1. Hospital course of fever and output of diarrhea in 37 patients with typhoid fever who completed at least nine days of antibiotic treatment and survived wilh no intestinal perforation. Brackets indicate the SE.

in volunteers [27] who had the disease and did not develop diarrhea; this difference suggests that naturally acquired typhoid fever shows a lower threshold of resistance for diarrhea that could be caused by an association with intestinal diseases or by nutritional factors in developing countries. The wide range in the severity of diarrhea in our patients, from three to 30 stools passed per day and from 4 to 172 ml of diarrheal fluid/kg of body weight passed during the first day in the hospital, suggests also that these patients were not preselected for severe diarrhea and were an appropriate group for the study of the pathogenesis of diarrhea in typhoid fever.

Our results confirm the findings of Stoll et al. [20] that the diarrhea of patients with typhoid fever is watery and contains leukocytes. Our results further establish that the stools contained leukocytes over a wide range of counts, with a mean of 4,950 cells/mm^3, and a predominance of PMNLs. Red blood cells were less regularly present and were usually in smaller numbers than white blood cells. The stools contained high concentrations of protein and had a mean acidic pH of 6.1. The overall fecal composition in these patients indicates that diarrhea associated with typhoid contrasts with that associated with cholera by showing more fecal leukocytes and more protein, containing lower concentrations of sodium, chloride, and bicarbonate, but containing higher concentrations of potassium. The diarrhea of these patients with typhoid fever contrasts with that associated with shigellosis in that it is generally more watery, does not cause frank dysentery except in the few patients who passed grossly bloody stools, and contains lower counts of fecal leukocytes (P. S., unpublished observations).

The earlier view of diarrhea associated with typhoid was that diarrhea developed late in the course of the disease. Stuart and Pullen [14] found that diarrhea occurred usually on the eighth or ninth day of illness, and Ikeme and Anan [17] described diarrhea occurring two weeks or more after the onset of fever. The onset of diarrhea at this later period, when ulceration in the ileum is advanced and the complications of perforation and hemorrhage typically occur, implies that local inflammation in the ileum causes the diarrhea. Our findings, on the other hand, suggest that diarrhea occurred earlier than two weeks in most patients. The histories of our patients revealed that the mean prehospital duration of fever and diarrhea was 9.5 days and 5.8 days, respectively, a finding indicating that diarrhea started at an average time of ^3.5 days after the onset of fever. Furthermore, the diarrhea in these patients resolved during treatment with chloramphenicol al a rate that approximately paralleled the resolution of fever. This close temporal association of fever and

diarrhea in typhoid fever suggests that the mechanism of diarrhea may be related to that of fever. Fever is a symptom produced by the release of endogenous pyrogen, or interleukin-1, from mononuclear phagocytic cells and is mediated through prostaglandins [28, 29]. The origin of diarrhea in patients with typhoid fever could have a similar pathogenesis; the presence of leukocytes in the stool, including mononuclear leukocytes, supports this hypothesis as a possible mechanism.

Although the pathogenesis of typhoid fever and nontyphoid salmonellosis differ markedly by virtue of the ability of S. *typhi* to establish a systemic intracellular infection of the reticuloendothelial cells, there is also local intestinal disease in typhoid fever that typically results in hyperplasia of Peyer's patches in the ileum with ulceration of overlying tissue. Ulcers in the proximal colon are also common. Some of the local mechanisms operative in nontyphoid salmonella! gastroenteritis may be active in patients with typhoid diarrhea. The pathogenesis of diarrhea caused by *Salmonella typhimurium* has been studied experimentally in animals. Rats infected with 5. *typhimurium* developed net water secretion in the ileum, but colonic function was not significantly changed [30, 31]. Rhesus monkeys infected with 5. *typhimurium* developed a net secretion of water, sodium, and chloride in the jejunum, ileum, and colon [32], Net fluid secretion in the colon was always observed together with a marked colitis that was characterized by microabsecesses and epithelial disruptions. In the ileum of rabbits infected with *Salmonella,* invasion of the tissue by bacteria followed by intense inflammation was associated with the net fluid secretion and active ion secretion. Increases of adenyl cyclase in mucosal cells measured by Giannella et al. [33J indicated a role for cyclic AMP in experimental salmonellosis; a blockade of the fluid production and rise in adenyl cyclase by treatment with indomethacin suggested also that the diarrhea was mediated by prostaglandins. Further studies of experimental infection with *Salmonella* in the ileum of the rabbit showed thai the protein content of infected ileal loops was increased, but filtration of plasma proteins into the loop was not demonstrated [34). This finding suggests that increased stool protein in typhoid fever probably does not result primarily from exudation of plasma protein but may be a manifestation of tissue injury and inflammation.

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