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Tetracycline in the Treatment of Severe Cholera Due to *Vibrio cholerae* O139 Bengal

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ABSTRACT

Vibrio cholerae O139 synonym Bengal, recognized in 1993, is the second member in the list of about 200 serogroups of *V. cholerae* with epidemic and pandemic potential. Although replacement of fluids and electrolytes remains the cornerstone in the management of cholera, antimicrobial therapy can significantly shorten the duration of diarrhoea, and reduce stool volume and requirements of rehydration fluids. The role of antimicrobial therapy on the natural course of the disease caused by this relatively new pathogen has not been systematically assessed. A randomized, double-blind, placebo-controlled clinical trial was conducted to evaluate the efficacy of tetracycline in the treatment of adults with severe cholera due to *V. cholerae* O139 Bengal. Forty-three adult males with severe cholera were randomly allocated to receive either 500 mg of tetracycline (n=21) or placebo (n=22) for three consecutive days. Demographic and clinical characteristics of these patients on admission were comparable. Tetracycline therapy was associated with significantly reduced total median (inter-quartile range) stool volume [216.48 (90.18-325.22) mL/kg vs 334.25 (215.12-537.64) mL/kg; p=0.001], higher rates of clinical cure (81% vs 27%; p<0.001), and shorter median (inter-quartile range) duration of diarrhoea [32 (24-48) hours vs 80 (48-104) hours; p<0.001]. The mean±(SD) requirement of intravenous fluid was not significantly different between the two groups [146.42±42.12 mL/kg vs 150.44±27.21 mL/kg; p=0.70]. The median (inter-quartile range) duration of faecal excretion of *V. cholerae* O139 was significantly shorter in the tetracycline group than the placebo group [1(1-2) day vs 5 (3-6) days; p<0.001]. The results of the study indicate that tetracycline therapy is clinically useful in the treatment of severe cholera due to *V. cholerae* O139 Bengal.

Key words: Cholera; *Vibrio cholerae*; *Vibrio cholerae* O139 Bengal; Tetracycline; Antibiotics; Drug therapy; Randomized controlled trials; Double-blind method; Bangladesh

INTRODUCTION

Clinical spectrum of infection due to *Vibrio cholerae* varies widely–ranging from asymptomatic infection to severe disease which is characterized by frequent passage of voluminous watery stools, vomiting, dehydration, hypovolaemic shock, and sometimes, death (1). Until recently, *V. cholerae* O1 has been the only recognized serogroup among 138 serogroups causing epidemic cholera (2,3); other serogroups have been associated with sporadic diarrhoea without causing epidemics (4). Recently, a new serogroup of *V. cholerae*, designated *V. cholerae* O139 synonym Bengal (5), has been reported to cause large epidemics of severe dehydrating diarrhoea in India and Bangladesh (6-8). However, the pathophysiologic mechanism and clinical course of the disease, caused by *V. cholerae* O139 Bengal, have not been characterized well but reported to be similar to those of *V. cholerae* O1 (9-11).

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Management of dehydration and antimicrobial treatment remain the mainstay in the treatment of cholera. Studies in the early 1960s have shown that antimicrobial therapy reduces the duration of illness, stool volume and requirement of rehydration fluids, and shortens the duration of faecal excretion of *V. cholerae* O1 (12-14). However, the therapeutic effects of antibiotics on the natural course of cholera caused by *V. cholerae* O139 have not been evaluated. We, therefore, conducted a randomized, double-blind, placebo-controlled clinical trial to evaluate the efficacy of tetracycline in the treatment of adults with severe cholera due to *V. cholerae* O139 Bengal.

MATERIALS AND METHODS

Estimation of sample size

With reference to total stool volume in cholera patients not treated with antimicrobial agents (24), a pre-trial sample size of 21 patients in each group was estimated to detect a 50% reduction in total stool volume treated with tetracycline to give a power of 80% at a significance level of 5%.

Selection of patients

The study was conducted at the Clinical Research and Service Centre (CRSC) of ICDDR,B: Centre for Health and Population Research during September-November 1993. The CRSC provides treatment to 100,000-120,000 diarrhoeal patients each year. Every 4th male patient, aged 18-60 years, seeking treatment at the CRSC with a history of watery diarrhoea of less than 12 hours with some or severe dehydration as per the WHO guidelines (17) and presence of motile vibrios on dark-field microscopic examination of stool (15), not receiving any antimicrobial therapy for the current illness, was considered eligible for this study. Patients with clinical evidence of any concurrent illnesses were excluded. Written informed consents were obtained from patients for participation in the study.

Management of patients

On admission to the study, patients were weighed and placed on a 'Cholera Cot' for collection of stool unmixed with urine (16). Medical history was obtained either from the patients or from attending relatives. Physical examinations, including assessment of dehydration (17), were performed, and the findings were recorded. Patients were then rehydrated with a polyelectrolyte solution (sodium 133 mMol/L, potassium 13 mMol/L, chloride 98 mMol/L, and bicarbonate 48 mMol/L), following the WHO guidelines (18,19). Rice-based oral rehydration solution (ORS) was used as the maintenance fluid (20) during the course of their illness. The same intravenous polyelectrolyte solution was used for patients whose hydration status could not be maintained with oral rehydration fluid due to excessive vomiting and/or very high rates of purging. Fluid intake (intravenous and oral rehydration fluids, plain water, and milk) and output (volume and weight of liquid stools, urine, and vomitus) were recorded for the initial rehydration phase and for the subsequent 8-hourly periods of the study. Radial pulse and respiration rates, oral temperature, and blood pressures were recorded on admission and 8-hourly during the study. The patients were re-weighed after initial rehydration and daily thereafter.

Concentrations of serum and stool electrolytes, and serum-specific gravity were determined before initial rehydration of the patients. Stool and rectal swab cultures were performed before initiation of antimicrobial therapy and at 24-hour intervals from the time of administration of the study drug.

The patients were hospitalized until recovery and faecal culture for *V. cholerae* O139 were negative for at least two consecutive days.

Laboratory procedures

Stool samples and rectal swabs were inoculated directly onto tellurite-taurocholate-gelatin agar (TTGA) media after six hours of enrichment in alkaline peptone water. Suspected colonies of *V. cholerae* were isolated and identified following standard bacteriologic procedures (21). Specific monoclonal antibodies against *V. cholerae* O139 were used for slide agglutination test (22), and a highly sensitive and specific coagglutination test was also used (23) to confirm diagnosis of *V. cholerae* O139 Bengal.

Randomization and patients compliance

After admission, the patients were randomized to receive either 500 mg of tetracycline capsules or placebo every six hours for three consecutive days (total of 12 doses). For randomization, the patients were assigned a sequential number which had been pre-assigned to either of the therapies. A computer-generated randomization plan was used for the purpose. A senior member of the staff of Clinical Sciences Division of ICDDR,B, who was not directly involved in the study, prepared the random list and kept it confidential. To conceal treatment allocation,

the pharmacist provided medication corresponding to the identification number to the study nurse when a subject was ready to be enrolled. The study nurses offered assigned treatment to patients and ensured swallowing of the capsules by patients. Administration of each dose of the study drug was recorded.

Definitions

We defined 'diarrhoea' as passage of at least one liquid stool per day, irrespective of volume. A 'formed stool' was defined as the one that retains its shape; a 'soft stool' was defined as the one that sticks to and takes the shape of the container but cannot be poured like water; and the 'liquid stool' as the one that can be poured like water from one container to another. 'Duration of diarrhoea' was defined as the interval between administration of the first dose of study drugs and the end of the last 8-hour period when a liquid stool has been passed, followed by no liquid stool during the next two 8-hourly periods. Patients were considered to have 'clinical cure' if diarrhoea stopped within 72 hours (3 days) of initiation of study drugs, and 'clinical relapse' was defined as initial resolution of diarrhoea followed by passage of liquid stool anytime during the study. 'Bacteriologic success' was defined as eradication of *V. cholerae* O139 from faeces within 72 hours after initiation of study drugs, and bacteriological relapse as a positive culture following the initial bacteriologic success. 'Unscheduled intravenous therapy' was defined as the condition when re-institution of intravenous fluid therapy was required at anytime after initial rehydration whether consequent to frequent vomiting or high purging rates or both along with re-appearance of clinical sign(s) of dehydration.

Statistical analysis

Edited data from 'Case Report Forms' were entered onto a personal computer using STATA software package (STATA statistical software: release 5.0. College Station, TX: Stata Corporation, 1997). Differences between the groups for normally distributed continuous variables were tested for significance using Student's *t*-test, and Willcoxon Rank-sum test was used for comparing continuous variables that were skewed. Chi-square test was performed to assess the significance of differences in proportions in the two study groups, and Fisher's exact test was performed when the predicted cell size in any group was less than 5. All tests were two-tailed, and the differences in values were considered significant if p was <0.05.

RESULTS

A total of 50 patients–25 in each group–were initially enrolled. Seven patients (4 in tetracycline group and 3 in placebo group) were excluded from analysis due to failure to isolate *V. cholerae* O139 from the faecal samples of 5 patients (3 in tetracycline group and 2 in placebo group) and incomplete study (2 patients). Of the total 43 evaluable patients, 21 received tetracycline and 22 received placebo.

Admission characteristics

Admission characteristics of the patients, including age, body weight, history of pre-admission duration of diarrhoea, duration of vomiting, anorexia, intake of ORS at home, and the dehydration status were comparable between the groups (Table 1).

Intravenous fluid required for initial rehydration

No significant difference (p=0.70) was observed between the groups regarding requirement of intravenous fluid during the initial rehydration period (Table 2).

Stool volume

No significant difference (p=0.57) in median stool output (mL/kg body weight) was observed during the first 24

Table 1. Admission characteristics of patients with cholera due to *V. cholerae* O139

Characteristics	Tetracycline group (n=21)	Placebo group (n=22)
Age (in years)	37.7±11.2	38.4±10.7
Body weight (kg)	43.01±4.4	41.4±5.2
History of pre-admission		
Duration of diarrhoea (hours)	8.1±2.2	8.2±2.5
Duration of vomiting (hours)	10.6±2.1	9.3±2.2
Anorexia, no. (%)	16 (76)	17(77)
Intake of ORS at home, no. (%)	15 (68)	19 (86)
Dehydration status		
Some, no. (%)	1 (5)	0
Severe, no. (%)	20 (95)	22 (100)

Values are mean±standard deviation, unless otherwise specified

Table 2. Clinical outcomes of patients

Outcomes	Tetracycline group (n=21)	Placebo group (n=22)	p value
Total volume (mL/kg) of intravenous fluid required for initial rehydration (mean±SD)	146.42±41.12	150.44±27.21	0.70
Stool volume (mL/kg body weight)			
0-24 hour(s)	192.58 (75.97-258.75)	203.03 (98.15-300.07)	0.57
25-48 hours	18.29 (6.2-32.0)	92.77 (59.60-124.69)	0.0007
49-72 hours	0 (0-0)	61.58 (25.99-114.30)	0.0001
Total stool volume during 0-72 hour(s)	216.48 (90.18-325.22)	334.25 (215.12-537.64)	0.019
Total volume of oral rehydration solution required during first 72 hours (mL/kg)	209.93 (119.92-272.93)	311.90 (245.54-405.63)	0.005
Unscheduled IV fluid required			
0-24 hour(s), No. (%)	9 (43)	9 (41)	0.965
25-48 hours, No. (%)	3 (17)	6 (27)	0.29
49-72 hours, No. (%)	0 (0)	5 (23)	0.02
Clinical cure by day 3, No. (%)	17 (81)	6 (27)	0.001
Bacteriologic success by day 3, No. (%)	19 (90)	8 (36)	0.001
Duration of diarrhoea (hours)	32 (24-48)	80 (48-104)	0.0001

Values are median (inter-quartile range), unless otherwise specified

hours after initiation of therapy (Table 2). However, significantly less stool output was observed in the tetracycline group during the second 24 hours of the study [18.29 (6.2-32.0) mL/kg vs 92.77 (59.60-124.69) mL/kg; p=0.0007] and onward. The total median (inter-quartile range) stool volume (mL/kg) over 72 hours after initiation of therapy was significantly less in the tetracycline group than in the placebo group [216.48 (90.18-325.22) mL/kg vs 334.25 (215.12-537.64) mL/kg, p=0.01].

Requirements of oral rehydration fluid

The total median (inter-quartile range) requirement of ORS required during the first three days was significantly lower in the tetracycline group than the placebo group [209.93 (119.92- 272.93) mL/kg vs 311.90 (245.54-405.63) mL/kg; p<0.001] (Table 2).

Unscheduled intravenous fluid

Proportion of patients who required unscheduled intravenous fluid (IV) during first and second study day after initiation of therapy was comparable between the groups (Table 2). On third study day significantly more patients in the placebo group required uncheduled IV than the tetracycline group: [0/21 in the tetracycline group vs 5/22 (23%) in the placebo group; p=0.02].

Clinical cure, bacteriologic success, and duration of diarrhoea

By day 3, the rate of clinical cure was significantly higher in the tetracycline group than in the placebo group (81% vs 27%; p<0.001), and bacteriologic success was also significantly higher in the tetracycline group than in the placebo group (90% vs 36%; p<0.0001) (Table 2). The median (inter-quartile range) duration of diarrhoea was also significantly shorter in the tetracycline group [32 (24-48) hours vs 80 (48-104) hours in the placebo group; p<0.001)].

Time of onset of therapeutic action

Pattern of stool output was analyzed to find out the earliest therapeutic effect of tetracycline on stool volume (Fig. 1). There was no difference in the stool volume between the groups during the first and the second eight hours. The earliest significant difference in median (inter-quartile range) stool output (mL/kg) was observed during the third eight hours (17-24 hours) [30.93 (10.28-44.33) mL/kg in the tetracycline group vs 54.70 (17.76-76.27) mL/kg in the placebo group; p=0.02].

Duration of faecal excretion of *V. cholerae* O139

The median (inter-quartile range) duration of excretion of *V. cholerae* O139 in stool was significantly shorter in the tetracycline group compared to the placebo group [1 (1-2) day vs 5 (3-6) days; p<0.001] (Fig. 2). Two (9.5%) patients in the tetracycline group excreted *V. cholerae* O139 in their stools up to five days compared to 13 (59%) patients in the placebo group (p<0.001) during the same period (not shown in Fig. 2), two (9%) patients in the placebo group had a positive stool/rectal swab culture up to 15 days, and one continued to excrete *V. cholerae* O139 up to 21 days (not shown in Fig. 2). These two

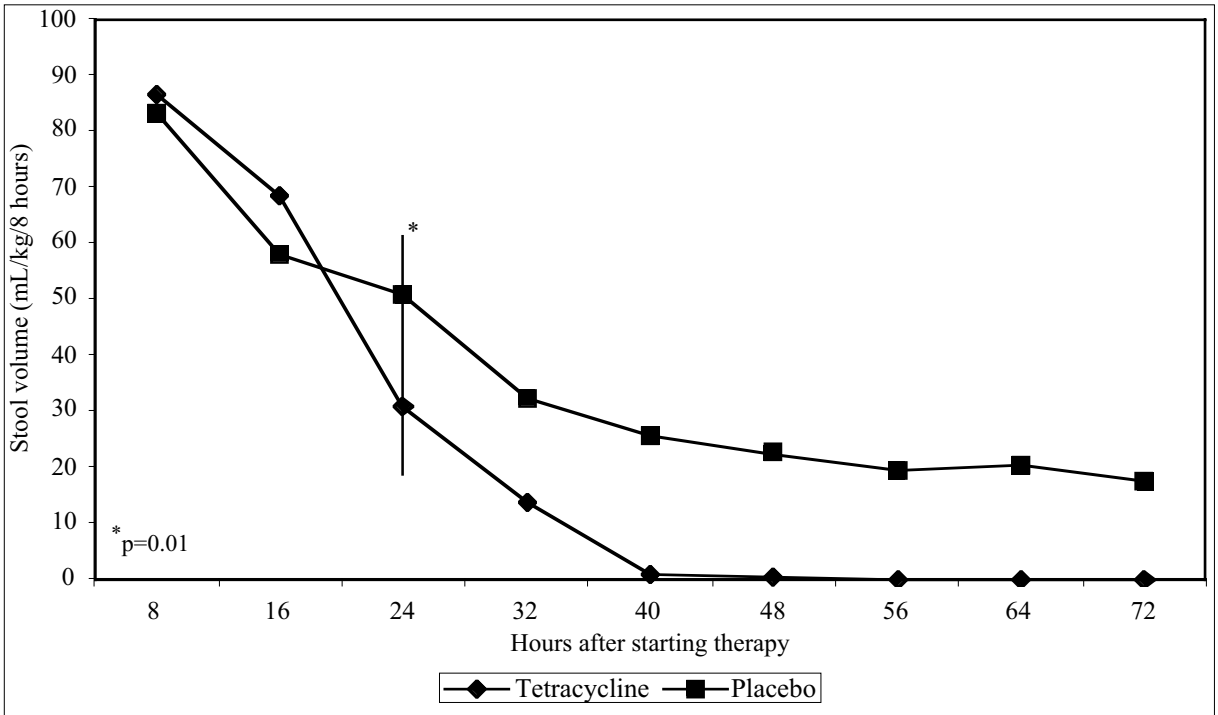

Fig. 1. Eight-hourly stool output of patients during the first 72 hours of study

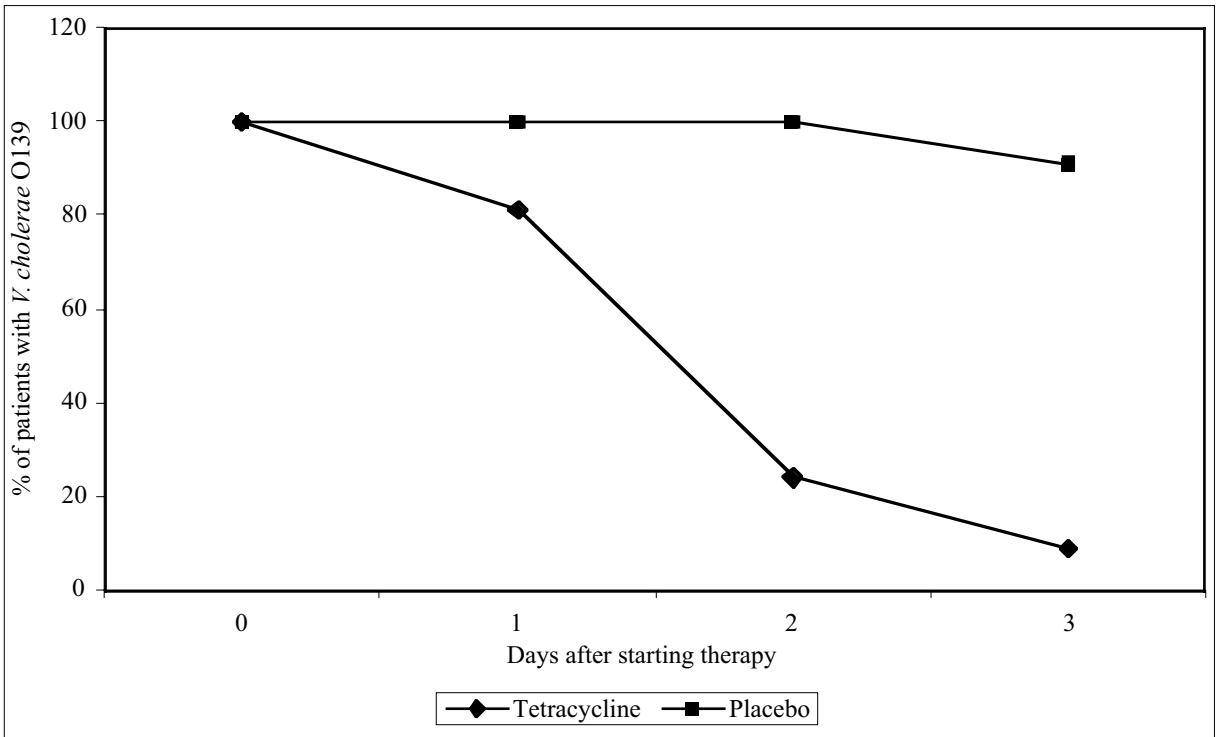

Fig. 2. Excretion of *V. cholerae* O139 in stool during first three consecutive days of study

patients were, however, symptom-free after third study day. We did not observe any relapse or reinfection during the study period. All strains of *V. cholerae* O139 were sensitive to tetracycline as determined by the disc-diffusion method.

DISCUSSION

This is the first study assessing the efficacy of tetracycline on the natural course of severe cholera due to *V. cholerae* O139 infection among adults. We have included a placebo group because the natural course of the disease caused by *V. cholerae* O139 is unknown, and the impact of antimicrobial therapy on the natural course has not been assessed. Second, earlier studies in cholera due to *V. cholerae* O1 could not demonstrate any risk associated with placebo administration; complications and deaths from this disease can be avoided through maintenance of hydration and continuation of feeding.

The results of the study clearly demonstrated that tetracycline significantly reduced the volume of stools and requirements of oral rehydration fluids, enhanced clinical cure, and reduced duration of diarrhoea and faecal excretion of *V. cholerae* O139. The study further demonstrated that an effective response to tetracycline in the treatment of severe cholera due to *V. cholerae* O139 took place after 16 hours of initiation of therapy. Similar to the findings of an earlier study in cholera due to *V. cholerae* O1 (25), we observed the impact of therapy within the first 24 hours of initiation of an antimicrobial therapy. The magnitude of reduction in the duration of diarrhoea (60%), total stool volume during the first 72 hours (35%), and median requirements of oral rehydration fluids (33%) associated with antimicrobial therapy in our study are similar to the findings of earlier studies on antimicrobial therapy for cholera due to *V. cholerae* O1 (14,26,27).

In our study, tetracycline therapy also shortened the duration of faecal excretion of *V. cholerae* O139 significantly. *V. cholerae* O139 became culture-negative from faeces of 91% of patients in the tetracycline group within 72 hours after initiation of therapy, while 50% of patients in the placebo group excreted the organism beyond five days. In our study with tetracycline, we did not observe any bacteriological relapse.

The value of tetracycline in the treatment of cholera due to *V. cholerae* O1 is well-established. Tetracycline acts as an adjunct to intravenous fluid and electrolyte replacement, and significantly reduces the duration of diarrhoea and vibrio excretion, thereby diminishing the total amount of intravenous fluid (12). Although in our study we did not observe any differences in the volume of intravenous fluid required for rehydration during the first 48 hours after initiation of therapy, the requirement of unscheduled IV was significantly more on third study day ($p=0.02$), and consequently, significantly more intravenous fluids ($p=0.01$) were required by patients in the placebo group on the third day of study.

Oral administration of 500 mg of tetracycline six hourly for 72 hours produced excellent results in the treatment of severe cholera due to *V. cholerae* O139. However, there are two important limitations of our study that make general applications of the results difficult. First, we studied more severe disease in adult males. Antimicrobial therapy is recommended for the treatment of severe cholera, and the role of antimicrobial therapy for milder cholera is less clear; however, milder cholera cases would be less likely to be recognized clinically and could be effectively managed by oral rehydration therapy and continued feeding. It is also likely that tetracycline will be effective in women and children with similar infections. Second, tetracycline may not be recommended in young children (who are the major victims of cholera in endemic countries) and in pregnant women. Identification of safe agents for the management of cholera in these populations would be important.

The emergence of tetracycline-resistant strains of *V. cholerae* O1 has been reported from several countries, including Bangladesh (28). Since cholera caused by O1 and O139 serogroups are clinically indistinguishable (29) in areas where *V. cholerae* O1 and *V. cholerae* O139 with different patterns of susceptibility to tetracycline coexist (as in Bangladesh), the use of empiric tetracycline therapy will depend on the local susceptibility patterns of *V. cholerae*. For the management of tetracycline-resistant cholera, it would be useful to identify alternative agents to which both the serogroups of *V. cholerae* are susceptible. One such drug used in the management of cholera in children and pregnant and lactating women is erythromycin (30). However, clinical trials are necessary to determine if erythromycin is also effective in the treatment of cholera due to *V. cholerae* O139, although it is highly likely that it will be.

In summary, in patients presenting with severe cholera due either to *V. cholerae* O1 or to *V. cholerae* O139, there is critical loss of body water and electrolytes, and significant reduction in stool volume can be observed

24 hours after treatment with an effective antimicrobial agent. Thus, appropriate management of fluid and electrolyte remains the cornerstone in the management of severe cholera, and antimicrobial therapy would remain as an important adjunct therapy.

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