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Independent prognostic factors for fatality in patients with invasive *Vibrio cholerae* non-O1 infections

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To identify independent prognostic factors for fatality, 73 patients with a total of 75 episodes of invasive *Vibrio. cholerae* non-O1 infections treated from July 1998 through October 2001 at 2 medical centers were retrospectively studied. The demographic, laboratory, and clinical information of these patients were collected and analyzed. The overall mortality rate was 36%. Multivariate analysis revealed that severe liver cirrhosis (p=0.003; odds ratio [OR], 14.12, with 95% confidence interval [CI] 2.49-79.91), malignancy (p=0.034; OR, 3.9, with 95% CI 1.11-13. 7), and steroid use (p=0.011; OR, 12.37, with 95% CI 1.79-85.49) were independent risk factors for fatality. These findings suggest that patients at high risk of fatality should be hospitalized and aggressively treated when *V. cholerae* non-O1 infections develop, and that public education on how to avoid exposure to *V. cholerae* non-O1 is important for the high-risk population.

Key words: Liver cirrhosis, malignancy, risk factor, steroid *Vibrio cholerae* non-O1 infection

Vibrio cholerae non-O1 is a globally distributed non-halophilic gram-negative bacillus, which often causes sporadic self-limited diarrheal disease [1,2]. However, in immunocompromised patients, invasive infections such as bacteremia, peritonitis, cholangitis, and necrotizing fasciitis caused by this pathogen were not uncommon [3-10]. As invasive *V. cholerae* non-O1 infections result in a high mortality in immuno-compromised patients [3,4], timely and effective treatment for this disease is essential. Unfortunately, little is known regarding the fatality risk factors in patients with invasive *V. cholerae* non-O1 infections. To better define these risk factors, we retrospectively studied cases of invasive *V. cholerae* non-O1 infections treated between June 1998 and October 2001 at one medical center in northern Taiwan and one in southern Taiwan to identify independent prognostic factors for fatality.

Materials and Methods

Records of *V. cholerae* non-O1 isolated from normally sterile sites between June 1998 and October 2001 at the Clinical Microbiology Laboratories of Chang Gung Memorial Hospital, Lin-Kuo Medical Center (3800-bed facility) and Chang Gung Memorial Hospital, Kaohsiung Medical Center (2500-bed facility) were used to compile a list of patients with invasive infections caused by this pathogen during this period. Because the pathogens were isolated from normally sterile sites, all of these patients were considered having invasive *V. cholerae* non-O1 infections. Medical charts of these patients were reviewed. Collections of demographic, laboratory, and clinical data included age, sex, signs and symptoms, underlying diseases and/or conditions, administered antibiotics, and clinical outcomes. Patients with blood cultures yielding *V. cholerae* non-O1 who had no other infection focus were considered to have primary bacteremia. Cirrhotic patients showing clinical peritoneal signs, with polymorphonuclear cells in ascites >250 cells/μL or whose ascites culture yielded *V. cholerae* non-O1, with or without additional positive blood culture, were considered suffering from spontaneous bacterial peritonitis. Patients with clinically related signs and symptoms and whose bile or blood culture yielded *V. cholerae* non-O1 were considered to have cholangitis. Patients with watery diarrhea and *V. cholerae* non-O1 yielded from their blood culture were considered to have gastroenteritis. Patients with clinically compatible signs and symptoms of soft tissue infection and *V. cholerae* non-O1 yielded from blood culture were considered to have cellulitis. Elderly patient is defined as ≥65 years of age. Severe liver

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cirrhosis was referred to that assessed, using modified Child-Pugh's scoring system, at Child C (Pugh's score ≥11 points) [11]. A patient with *V. cholerae* non-O1 infection was regarded as receiving appropriate antimicrobial therapy if at least one susceptible antibiotic was administered upon the patient's arrival at the hospital or upon the infection emergence in hospitalized patients. The susceptibility tests were performed on routine clinical service basis. In patients suffering from recurrent invasive *V. cholerae* non-O1 infection, each infection episode was regarded as a separate independent case. Each case of invasive *V. cholerae* non-O1 infection included in this study was categorized as either fatal or nonfatal. A fatality was defined as a case involving a patient with invasive *V. cholerae* non-O1 infection who died of all-cause during hospitalization.

Bacterial identification

V. cholerae isolates were identified based on the recognition of microscopically curved gram-negative bacteria with positive oxidase reaction, β-hemolysis on blood agar plate, yellowish colonies on thiosulfate citrate bile salts sucrose agar plate, susceptibility to 10 μg and 150 μg of vibriostatic agent O129 (2, 4-diamino-6, 7-diisopropylpteridine) (Rosco, Taastrup, Denmark), tolerability to 1% salt solution, and intolerability to 10% salt solution. Typical biochemistry profiles for *V. cholerae* included positive nitrate reduction, indole production, citrate utilization, D-Glucose utilization with acid production and without gas, Voges-Proskauer test, ornithine decarboxylase production, and lysine decarboxylase and negative arginine dihydrolase production and urea hydrolysis [12]. These characteristics were verified using the API-20E System (bioMérieux Vitek, Hazelwood, MO, US) or through traditional biochemistry reactions observed in test tubes. Serotyping of all strains was performed with the use of poly-O1 antiserum (Difco Laboratories, Detroit, MI, US).

In vitro antibiotic susceptibility testing

In vitro antibiotic susceptibility testing was performed using the Kirby-Bauer disk-diffusion method. Tested antibiotics included trimethoprim/sulfamethoxazole (1.25/23.75 μg per disk), ampicillin (10), piperacillin (100), cefazolin (30), cephalothin (30), cefamandole (30), cefuroxime (30), ceftriaxone (30), ceftizoxime (30), ceftazidime (30), cefepime (30), imipenem (10), aztreonam (30), gentamicin (10), amikacin (30), ciprofloxacin (5), and tetracycline (30). The performance procedures and breakpoint diameters for interpretation were in accordance with the National Committee for Clinical Laboratory Standards [13].

Statistical analyses

Because each episode of invasive infection was treated as an individual case, a patient included in this study could contribute more than one record to the analyses. Chi-squared test or Fisher's exact test was used to analyze dichotomous variables. Variables found to be statistically significant in univariate analyses were entered in multivariate analyses using a multiple logistic regression model to identify independent prognostic factors for fatality due to invasive *V. cholerae* non-O1 infections. A 2-tailed $p<0.05$ was considered statistically significant. All statistical analyses were performed using the SPSS software package, version 11.0 (SPSS Inc., Chicago, IL, US).

Results

During the 40-month study period, 75 individual episodes of *V. cholerae* non-O1 infection were diagnosed involving a total of 73 patients. All of these 75 individual episodes were included in this study. Among the 73 patients, 2 were found to have suffered a second episode of invasive *V. cholerae* non-O1 infection in the following year. One of them was a woman who had primary bacteremia in the initial episode, and peritonitis as well as gastroenteritis with secondary bacteremia in the second episode. The other was a man who had cellulitis and secondary bacteremia in the initial episode, and primary bacteremia in the latter episode.

There were 48 men and 27 women in the patient population, with ages ranging from 18 to 80 years (mean, 56.1 ± 14.5 years). Twenty-five (33.3%) patients were ≥65 years. All of the patients had underlying diseases and/or conditions which predisposed themselves to the development of invasive *V. cholerae* non-O1 infection (Table 1). A total of 62 (82.7%) cases had liver cirrhosis of different degrees of severity. Of the 62 cases, 43 (57.3%) cases were in patients with severe liver cirrhosis. Other leading underlying diseases and/or conditions, in descending order, were malignancy (33.3%), peptic ulcer (32%), biliary stones (29.3%), and diabetes mellitus (21.3%). Of the 75 cases, 23 (30.7%) had a diagnosis of gastroenteritis, 23 (30.7%) had peritonitis, 11 (14.7%) cholangitis, 9 (12%) cellulitis, and 27 (36%) primary bacteremia. *V. cholerae* non-O1 were isolated from blood in 69 cases, ascites in 15, and bile in 5. *V. cholerae* non-O1 was concurrently isolated from different sites of some patients; 13 cases had concurrent isolation of the microorganism

Table 1. Demographic data, underlying diseases and/or conditions, and diagnoses in patients with invasive *Vibrio cholerae* non-O1 infections, as well as comparisons between fatal and nonfatal groups

Characteristic (no. of cases)	No. of cases (%)		*p*
	Fatal	Nonfatal	
Age, years			0.307
≥65 (25)	11 (44.0)	14 (56.0)	
<65 (50)	16 (32.0)	34 (68.0)	
Sex			0.032
Male (48)	13 (27.1)	35 (72.9)	
Female (27)	14 (51.9)	13 (48.1)	
Underlying diseases			
Severe cirrhosis (43)	22 (51.2)	21 (48.8)	0.002
Malignancy (25)[a]	14 (56.0)	11 (44.0)	0.011
Peptic ulcer (24)	5 (20.8)	19 (79.2)	0.060
Biliary stones (22)	10 (45.5)	12 (54.5)	0.272
Diabetes mellitus (16)	6 (37.5)	10 (62.5)	0.888
Renal insufficiency (7)	1	6	0.209
Gout (3)	1	2	0.922
Other underlying diseases (7)[b]	3	4	0.691
Underlying conditions			
Antacid-use (30)	9 (30.0)	21 (70.0)	0.377
Steroid-use (13)	8 (61.5)	5 (38.5)	0.035
Previous operation (26)	11 (42.3)	15 (57.7)	0.407
Diagnoses			
Primary bacteremia (27)	9 (33.3)	18 (66.7)	0.718
Peritonitis (23)	13 (56.5)	10 (43.5)	0.014
Gastroenteritis (23)	8 (34.8)	15 (65.2)	0.884
Cholangitis (11)	2 (18.2)	9 (81.8)	0.183
Cellulitis (9)	4	5	0.574

[a]Including 21 cases of hepatoma, 2 cases of leukemia, and 2 cases of peri-ampullar cancer.
[b]One case each of bronchial asthma, Sheehan syndrome, previous cerebral vascular accident, chronic lung disease, congestive heart failure, alcoholism with malnutrition, and hemolytic anemia.

from both blood and ascites, while 1 case had isolates from both blood and bile. Secondary *V. cholerae* non-O1 bacteremia was diagnosed in 42 (56%) cases. Patients in these cases might have concurrent infection sites caused by this pathogen. Of these 42 cases with secondary *V. cholerae* non-O1 bacteremia, 23 had a diagnosis of gastroenteritis; 21 had peritonitis; and 7 each had cholangitis and cellulitis.

The clinical manifestations are summarized in Table 2. Fever (92%), chills (89.4%), and jaundice (74.7%) were the most common symptoms. Hemograms showed leukocytosis (>10 000 cells/μL) in 21 (28%) cases, leukopenia (<4000 cells/μL) in18 (24%), and marked anemia (hemoglobin <10 gm/dL) in 30 (40%).

Table 2. Clinical manifestations of patients with invasive *Vibrio cholerae* non-O1 infections (n = 75)

Symptom/sign	No. of cases (%)
Fever	69 (92.0)
Chills	67 (89.3)
Jaundice	56 (74.7)
Abdominal pain	45 (60.0)
Diarrhea	23 (30.7)
Skin manifestation	9 (12.0)

Results of *in-vitro* susceptibility tests on *V. cholerae* non-O1 isolates are shown in Table 3. Patients in 71 (94.7%) of 75 cases were given appropriate antimicrobial therapy immediately. Four different cirrhotic patients with *V. cholerae* non-O1 bacteremia did not receive appropriate antibiotic therapy immediately. The clinical courses of these 4 patients were as follows. One of these patients had underlying liver cirrhosis, graded Child B, and survived *V. cholerae* non-O1 peritonitis although a 7-day susceptible antibiotic treatment with piperacillin was not begun until the 4th day of hospitalization. Another patient with underlying liver cirrhosis, graded Child B, survived despite receiving no antibiotic treatment due to impression of viral gastroenteritis during hospitalization. The remaining 2 patients had severe liver cirrhosis, graded Child C, and died of overwhelming sepsis shortly after arrival at our emergency service, and did not receive antimicrobial therapy. Their blood cultures subsequently grew *V. cholerae* non-O1.

Table 3. *In-vitro* antimicrobial susceptibility of *Vibrio cholerae* non-O1 isolates

Antimicrobial agent (no. of tested isolates)	No. of susceptible isolates (%)
Trimethoprim/sulfamethoxazole (49)	37 (75.5)
Ampicillin (75)	59 (78.7)
Piperacillin (75)	72 (96.0)
Cephalothin or cefazolin (75)	69 (92.0)
Cefamandole or cefuroxime (75)	75 (100)
Ceftazidime, ceftizoxime, or ceftriaxone (75)	75 (100)
Cefepime (19)	19 (100)
Imipenem (68)	68 (100)
Aztreonam (27)	27 (100)
Gentamicin (75)	75 (100)
Amikacin (75)	75 (100)
Ciprofloxacin (74)	74 (100)
Tetracycline (3)	3

A total of 27 (36%) of 75 cases of invasive *V. cholerae* non-O1 infections were fatal. Of the 27 fatal cases (concurrent infection sites caused by this pathogen may have been found), 13 had a diagnosis of peritonitis, 8 had gastroenteritis, 2 had cholangitis, 4 had cellulitis, and 9 with primary bacteremia.

Univariate analyses revealed significant differences between fatal and nonfatal patient groups in variables including sex, severe liver cirrhosis, malignancy, steroid use, and peritonitis (Table 1). Independent prognostic factors for fatality in patients with invasive *V. cholerae* non-O1 infections on multivariate analyses included severe liver cirrhosis (p=0.003; odds ratio [OR], 14.12, with 95% confidence interval [CI] 2.49-79.91), malignancy (p=0.034; OR, 3.9, with 95% CI 1.11-13.7), and steroid use (p=0.011; OR, 12.37, with 95% CI 1.79-85.49).

Discussion

The 36% fatality rate in patients with invasive *V. cholerae* non-O1 infection in this study is comparable to those previously reported, which varied from 23.8% to 61.5% [3,4]. Considering the high fatality rate, a hospitalization policy coupled with aggressive treatment is warranted for patients with invasive *V. cholerae* non-O1 infections. However, such a policy requires increased health care resources, which may not be readily available in the current climate of tightening medical budgets and health care regulations. Given these practical constraints, identifying patients with invasive *V. cholerae* non-O1 infections who have the greatest risk of fatality may enable the design of less costly case management approaches, and allow people with specific risk factors of fatality to be educated preemptively regarding ways to avoid exposure to *V. cholerae* non-O1.

The combination of a well developed aquaculture industry and tropical/subtropical climate in Taiwan make the island environmentally favorable for the proliferation of *V. cholerae* non-O1 [14]. As a result, it is impossible to substantially reduce the burdens of this island-wide-distributed pathogen in Taiwan. Consumption of fresh and saltwater fish and crustaceans is popular in Taiwan, and the food is sometimes served not fully cooked in Chinese-style delicacies. This preference and practice puts Taiwanese at high risk of *V. cholerae* non-O1 infection. Many Taiwanese take antacid concurrently with all medications because of misinformation that it provides stomach protection. This practice may also facilitate the entrance of *V. cholerae* non-O1 into the body due to reduced gastric acidity, which was reported to be a risk factor for acquisition of *V. cholerae* through the gastrointestinal tract [15]. Cutaneous injuries from outdoor activity during flooding in the typhoon season in Taiwan may offer another portal of entry for *Vibrio* spp. Remarkably, 17.3% of the patient population in this study had a history of steroid use. Illegal steroid-containing herbal drugs have long been widely used in rural communities in Taiwan. As a result, misuse of steroids is a frequent cause of immunoincompetence in Taiwan. All patients with fatal invasive *V. cholerae* non-O1 infections in this series had severe illness and underlying immunoincompetence. It is unclear whether the superimposing invasive *V. cholerae* non-O1 infection *per se* or the infection-associated further deterioration of the preexisting severe illness contributes to fatality of our patients. Only a case-control study can disclose the contributable fatality of invasive *V. cholerae* non-O1 infections in such vulnerable patients [16].

To our knowledge, this is the first reported study specifically designed to identify risk factors for fatality in invasive *V. cholerae* non-O1 infections. Liver cirrhosis, malignancy, and steroid use were found to

be independent risk factors for fatality in invasive infections caused by this pathogen. The detailed mechanisms involved in severe invasive *V. cholerae* non-O1 infections are not fully understood. However, it is noteworthy that liver cirrhosis is a poor prognostic factor in invasive infections caused by *Vibrio vulnificus* or *Aeromonas* spp. [17-20], 2 pathogens with a close taxonomical relation to *V. cholerae*. The survival of *V. vulnificus* in the whole blood of patients with chronic liver disease correlated with increased serum ferritin levels and decreased phagocytic activities [21]. Abnormally elevated serum levels of iron and estradiol are commonly found in severely cirrhotic patients [21-25]. Iron overload in serum impairs the phagocytic activity of neutrophils of both humans and animals [21, 22,26], and elevated levels of serum cortisol and estradiol are each capable of reducing phagocytic activities of neutrophils and alveolar macrophages of animals [27,28]. Further study is needed to determine whether or not the aforementioned mechanisms play important roles that may lead to catastrophic outcomes in patients with invasive *V. cholerae* non-O1 infections. Nonetheless, understanding of the independent prognostic factors for fatality in invasive *V. cholerae* non-O1 infections is of particular importance in Taiwan because liver cirrhosis, cirrhosis-related hepatoma, and steroid use have long been prevalent on the island.

In conclusion, clinicians should be alerted about potential cases of invasive *V. cholerae* non-O1 infections. Patients at high risk for fatality should be hospitalized and aggressively treated once *V. cholerae* non-O1 infection is diagnosed. Public education programs on how to avoid exposure to *V. cholerae* non-O1 are extremely important for people in high-risk categories.

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