

LIVER FAILURE AND DEATH AFTER EXPOSURE TO MICROCYSTINS AT A HEMODIALYSIS CENTER IN BRAZIL

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ABSTRACT

Background Hemodialysis is a common but potentially hazardous procedure. From February 17 to 20, 1996, 116 of 130 patients (89 percent) at a dialysis center (dialysis center A) in Caruaru, Brazil, had visual disturbances, nausea, and vomiting associated with hemodialysis. By March 24, 26 of the patients had died of acute liver failure.

Methods A case patient was defined as any patient undergoing dialysis at dialysis center A or Caruaru's other dialysis center (dialysis center B) during February 1996 who had acute liver failure. To determine the risk factors for and the source of the outbreak, we conducted a cohort study of the 130 patients at dialysis center A and the 47 patients at dialysis center B, reviewed the centers' water supplies, and collected water, patients' serum, and post-mortem liver tissue for microcystin assays.

Results One hundred one patients (all at dialysis center A) met the case definition, and 50 died. Affected patients who died were older than those who survived (median age, 47 vs. 35 years; $P < 0.001$). Furthermore, all 17 patients undergoing dialysis on the Tuesday-, Thursday-, and Saturday-night schedule became ill, and 13 of them (76 percent) died. Both centers received water from a nearby reservoir. However, the water supplied to dialysis center B was treated, filtered, and chlorinated, whereas the water supplied to dialysis center A was not. Microcystins produced by cyanobacteria were detected in water from the reservoir and from dialysis center A and in serum and liver tissue of case patients.

Conclusions Water used for hemodialysis can contain toxic materials, and its quality should therefore be carefully monitored. (N Engl J Med 1998;338:873-8.)

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IN the past 30 years, knowledge about end-stage renal disease and the use of maintenance hemodialysis for patients with the disease have both increased dramatically, and technological developments in dialyzer membranes, dialysis machines, and vascular access have made hemodialysis a common procedure. Nonetheless, it remains potentially hazardous, because of mechanical malfunctions and human error.¹ Hemodialysis may be especially hazardous in places where municipal water treatment or appropriate infection-control practices are inadequate.² Furthermore, water used for hemo-

dialysis may contain various harmful agents, including toxins produced by naturally occurring cyanobacteria (i.e., blue-green algae). These toxins can have anticholinesterase activity (e.g., anatoxin-a(s)) or cause hepatic injury (e.g., microcystins and nodularins) in animals that ingest large quantities of contaminated water.

During the period February 17 to 20, 1996, patients at a dialysis center (dialysis center A) in Caruaru, Brazil, had visual disturbances, nausea, and vomiting associated with hemodialysis. From February 20 through March 6, 1996, 12 patients died of seizures or hemorrhages attributed to acute liver failure. On March 7, the secretary of health for the state of Pernambuco was notified, an epidemiologic investigation was begun, and dialysis center A was closed. By March 24, a total of 26 patients had died of liver failure, approximately 20 others were hospitalized, and the Centers for Disease Control and Prevention had been invited to assist with the investigation. This report describes the results of that investigation.

METHODS

Case Definition

A case patient was defined as any patient who underwent hemodialysis in Caruaru at dialysis center A or the city's other dialysis center, dialysis center B, during February 1996 and who had acute liver failure. Liver failure was defined as a serum conjugated bilirubin concentration ≥ 1.0 mg per deciliter ($17.1 \mu\text{mol}$ per liter) or a serum aspartate aminotransferase concentration ≥ 68 U per liter in a patient who had previously documented normal serum liver-enzyme concentrations or as death from acute liver failure, as indicated on a death certificate.

Non-case patients were patients who underwent dialysis in Caruaru during February 1996 and who did not have liver failure.

Cohort Study

To identify case patients and determine risk factors for liver failure and death, we compared the patients who underwent dialysis

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at center A during February 1996 with the patients who underwent dialysis at center B. In April 1996, all the available patients and the relatives of patients who had died were interviewed. All medical and dialysis records were reviewed. Laboratory results, including white-cell counts, serum liver-enzyme and bilirubin values, and coagulation studies, were obtained for each patient for the period February to April 1996. Autopsy reports were reviewed to determine the causes of death of the patients who had died. Variables examined in the cohort study included age, underlying diseases, history of hepatitis B or hepatitis C infection, the timing of dialysis, the room number, and the number of the dialysis machine used.

Observational Studies

Procedures for water treatment at the municipal water-treatment plant and at both dialysis centers were reviewed. Personnel responsible for water treatment and transport were interviewed. Procedures for disinfecting the dialysis machines, reprocessing the dialyzers, and administering hemodialysis were reviewed, and the practices observed.

Toxicologic Studies

Samples of water from Tabocas Reservoir (Caruaru's water source), the municipal water-treatment plant, a truck used to transport water to dialysis center A, and multiple sites at both dialysis centers were collected for toxicologic evaluation. Dialysis water-treatment devices used at center A during the outbreak period, serum samples from some of the patients who underwent dialysis at both centers, and samples of liver tissue from some of the patients who died were obtained for analysis.

Water samples, methanol extracts of dialysis water-treatment filters, and serum and liver-tissue samples from patients were tested for microcystins by high-performance liquid chromatography, enzyme-linked immunosorbent assay, protein phosphatase-inhibition assay, and mass spectrometry.³

All the water samples were extracted, concentrated, and analyzed for the presence of pesticides, including organophosphates, by personnel of the Food and Drug Administration's National Center for Toxicological Research, in Jefferson, Arkansas.

Pathological Studies

Liver tissue was obtained at autopsy from 16 patients and was examined by light and electron microscopy.

Statistical Analysis

The results were analyzed with the use of Epi Info software.⁴ Categorical variables were compared by using chi-square or Fisher's exact two-tailed tests. Continuous variables were compared by using the Kruskal-Wallis rank-sum test or Student's *t*-test, and probability values were determined.

RESULTS

Cohort Study

During February 1996, 130 patients underwent hemodialysis at dialysis center A and 47 patients underwent hemodialysis at dialysis center B. Eighty percent resided in rural areas within 250 km of Caruaru. The patients' underlying diseases were primarily hypertension, acute glomerulonephritis, and chronic pyelonephritis. There were no significant demographic differences between the patients from center A and those from center B. The median age of the patients was similar at the two centers (40 years at center A and 45 years at center B, $P=0.22$), and both centers had similar percentages of men (58

percent and 64 percent, respectively; $P=0.57$). At both centers, dialysis was administered by a conventional hemodialysis system, with low-flux polysulfone membranes. The patients were treated for four hours three times per week, with a blood-flow rate of 300 ml per minute and a dialysate-flow rate of 500 ml per minute. Dialyzers were reused at both centers until there was a reduction of ≥ 20 percent in fiber-bundle volume or they had been used 40 times. At each center, dialyzers were used an average of 18 times. The underlying nutritional status of the patients at both centers was poor. The mean serum albumin concentration was 4.2 g per deciliter, the mean urea reduction was 63 percent, and the mean hematocrit was 23 percent; 35 percent of the patients at each center received erythropoietin therapy.

Laboratory or death-certificate data were available for 124 of the 130 patients (95 percent) from dialysis center A and 41 of the 47 patients (87 percent) from dialysis center B. One hundred one patients met the case definition; all underwent dialysis at center A. The attack rate was significantly higher at dialysis center A than at dialysis center B (101 of 124 patients [81 percent] vs. 0 of 47 patients, $P<0.001$). Of the 101 case patients, 50 had died of acute liver failure by September 15, 1996. No deaths have been attributed to liver failure since that date, although autopsy data regarding three case patients who died after September 1996 are not available. Two of 23 non-case patients (9 percent) at dialysis center A and 7 of 41 patients (17 percent) at dialysis center B died between February 20, 1996, and September 15, 1996, of causes unrelated to the outbreak; none died of liver failure. The median ages of case patients and non-case patients were similar (39 vs. 45 years, $P=0.11$). However, the case patients who died were significantly older than the case patients who survived (47 vs. 35 years, $P<0.001$).

The symptoms most frequently reported by interviewed case patients were visual disturbance (i.e., blurred vision, scotoma, or night blindness), nausea and vomiting, headache, and muscle weakness (Table 1). The majority of patients (42 of 83 [51 percent]) stated that their symptoms began between Saturday, February 17, and Tuesday, February 20, 1996. The visual symptoms resolved within approximately one week, but some patients continued to have muscle weakness and epigastric pain, even when the interviews were conducted in April.

We compared the median laboratory values for case patients and non-case patients before (i.e., February 1996) and after (i.e., March 1996) the probable exposure period (Table 2). In February 1996, all the patients tested had normal white-cell counts and serum concentrations of aspartate aminotransferase and total and conjugated bilirubin. In March 1996, however, the case patients had a small increase in white-cell count, a more-than-sevenfold increase in

TABLE 1. SYMPTOMS AND SIGNS REPORTED BY CASE PATIENTS IN CARUARU, BRAZIL, FEBRUARY 1996.

SYMPTOM OR SIGN	PROPORTION OF PATIENTS
	no. reporting/ no. evaluated (%)
Visual disturbance	86/96 (90)
Nausea and vomiting	69/95 (73)
Headache	60/93 (65)
Muscle weakness	51/95 (54)
Epigastric pain	43/94 (46)
Confusion	28/93 (30)
Bleeding*	25/92 (27)
Fever	20/93 (22)
Seizure	16/92 (17)

*Bleeding from the nose, gums, and gastrointestinal tract was reported.

TABLE 2. MEDIAN LABORATORY VALUES FOR CASE PATIENTS AND NON-CASE PATIENTS IN CARUARU, BRAZIL, FEBRUARY AND MARCH 1996.*

VARIABLE	NORMAL RANGE	CASE PATIENTS		NON-CASE PATIENTS	
		FEBRUARY	MARCH	FEBRUARY	MARCH
		(N=74)	(N=74)	(N=59)	(N=59)
White-cell count (per mm ³)	4.0–10.0	6.6	10.5†	6.2	7.1
Serum aspartate aminotransferase (U/liter)	10–34	12.5	87.4†	12.5	21.0†
Serum total bilirubin (mg/dl)	0.2–1.0	0.7	3.0†	0.8	0.8
Serum conjugated bilirubin (mg/dl)	0.5–0.3	0.4	2.2†	0.4	0.4
Prothrombin time (sec)	12–14	NA	17.5	NA	15.0

*To convert values for bilirubin to micromoles per liter, multiply by 17.1. NA denotes not available.

†P≤0.001 for the comparison with the value for February by t-test.

serum aspartate aminotransferase concentrations, a more-than-fourfold increase in serum concentrations of total and conjugated bilirubin, and prolonged prothrombin times. In contrast, the median values for white-cell counts and serum concentrations of aspartate aminotransferase and bilirubin in the non-case patients were within the normal ranges in February and March 1996. Although prothrombin times were abnormal in the non-case patients, the values were significantly lower than those in the case patients.

Among the other variables examined, only the dialysis schedule differed according to patient outcome. At dialysis center A, the patients were treated in six shifts, each scheduled at different times (i.e., Monday, Wednesday, and Friday mornings, afternoons, or nights; and Tuesday, Thursday, and Saturday mornings, afternoons, or nights). On the basis of the results of the chi-square test for heterogeneity, the six shifts differed significantly with regard to mortality rate (P=0.02) but not with regard to attack rate (P=0.10); the highest rates occurred among the patients who underwent dialysis on the Tuesday-, Thursday-, and Saturday-night shift (Fig. 1).

Observational Studies

Tabocas Reservoir is located approximately 40 km from Caruaru. A pipeline carries water from the reservoir to the municipal water-treatment plant (Fig. 2). There, alum was added to the water. After settling for two to three hours, the water was filtered through a large-particle sand filter, and then chlorine was added. This “finished” water was distributed through a water-distribution system to most of Caruaru, including dialysis center B. Dialysis center A was not included in this water-distribution system during the 1996 summer drought. Instead, dialysis center A received “unfinished” water, trucked from the municipal water-treatment plant twice daily. This water was treated with alum but not filtered or chlorinated. Occasionally, personnel at the water-treatment plant gave the truck driver chlorine to add to the water in his truck. However, there are no records to indicate whether or when chlorine was added during February and March 1996. After arriving at dialysis center A, the water was passed through a sand filter, a carbon-adsorption tank, a deionizer unit comprising cation and anion tanks, and a micropore filter before being used for hemodialysis. No chemicals were added to the water at the dialysis center. According to maintenance workers at dialysis center A, the carbon tank was changed approximately every six months, and the sand and micropore filters were changed approximately every three months; however, these devices had not been changed in the three months before the probable exposure, even though the center was receiving visibly turbid water from the delivery truck. On Saturday, February 17, 1996, after patients first reported symptoms, the sand and micropore filters at dialysis center A were changed. The carbon in the adsorption tank was changed on February 25, 1996.

Toxicologic Studies

No pesticides, including organophosphates, were found in samples of water from the reservoir, the municipal water-treatment plant, the truck used to transport water to dialysis center A, or the water-distribution systems of either dialysis center. Water



Figure 1. Attack Rate According to Dialysis Shift, Caruaru, Brazil, February 20 to September 15, 1996.

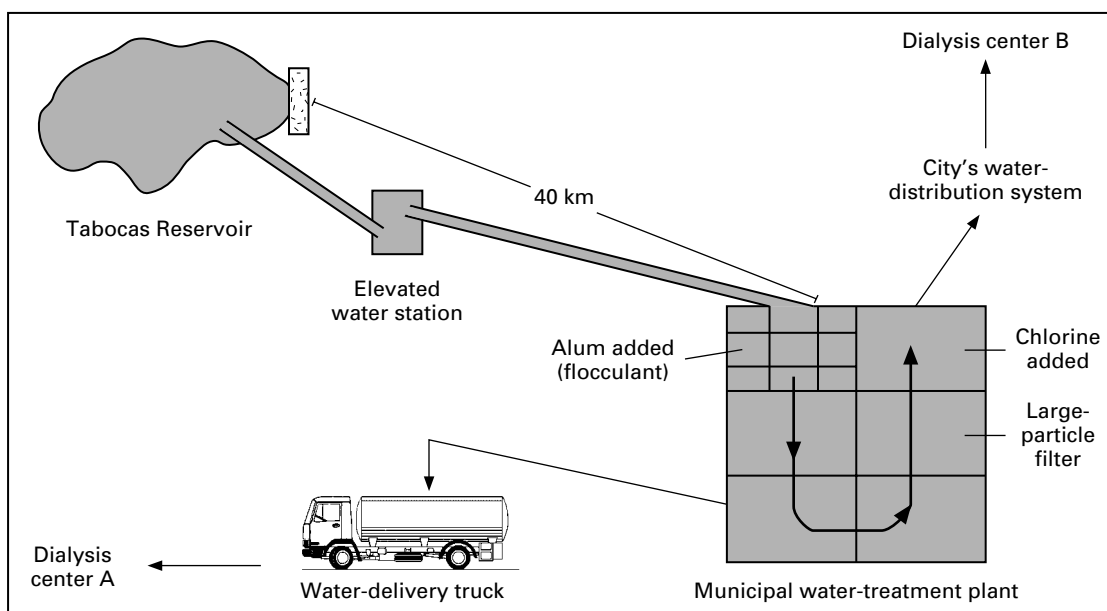


Figure 2. System of Water Treatment and Distribution in Caruaru, Brazil.

from the city's distribution system and treated water from dialysis center B contained no microcystins. By contrast, microcystins were detected by enzyme-linked immunosorbent assay in reservoir water, water obtained from the delivery truck, the water-holding tank and water-treatment devices (carbon and ion resins) at dialysis center A, and samples (serum and liver) taken before and after death from patients treated at this center. In March 1996, five persons in the region had been referred to the secretary of health for evaluation of dengue; serum samples from these persons tested as controls were negative for microcystins. The concentration of microcystins found in liver tissue from 17 case patients who died,

including 4 who were exhumed, ranged from 0.03 to 0.60 g per kilogram of liver tissue (median, 0.18).

Pathological Studies

Examination by light microscopy of liver tissue obtained at autopsy from 16 case patients revealed a very uniform pathological picture, with disruption of liver plates and cell deformity, extensive necrosis, apoptosis, severe cholestasis, cytoplasmic vacuolization, mixed-leukocyte infiltration, and occasional multinucleated hepatocytes; electron microscopy showed intracellular edema, mitochondrial changes, injuries to the rough and smooth endoplasmic reticula, lipid vacuoles, and residual bodies.

DISCUSSION

Water used for dialysis should be treated to remove potentially toxic materials.⁵ Certain substances in water are harmful to patients undergoing hemodialysis, who may be exposed to 150 liters of water per treatment. Adverse reactions have been traced to dialysis water containing elevated concentrations of aluminum,⁶⁻⁸ calcium,⁹ chloramines,^{10,11} copper,¹² fluoride,¹³ formaldehyde,¹⁴ hydrogen peroxide,¹⁵ sodium,¹⁶ sodium azide,^{17,18} sodium hypochlorite,¹⁹ and zinc.²⁰

In this outbreak, 101 of 124 patients (81 percent) who underwent dialysis at center A during February 1996 had acute liver injury, and 50 died. The highest attack and fatality rates were among patients who underwent dialysis during the Tuesday-, Thursday-, and Saturday-night shift. We hypothesize that a water shipment with a particularly high concentration of microcystins arrived during this shift. Patients first reported symptoms on Saturday, February 17, 1996. As a result of these reports, sand and micropore filters were changed that night. Multiple water shipments probably contained microcystins, and patients on all shifts were exposed to them. However, the filter changes and cleaning procedures performed after the February 17 night shift and the dilutional effect of additional water shipments may have resulted in lower exposure for the patients treated on other shifts.

Inadequate water treatment at both the municipal water plant and the dialysis center facilitated this outbreak. The water from the municipal plant contained microcystins not removed by the inadequately maintained treatment system at the dialysis center. Infectious diseases (e.g., viral hepatitis), hyperchlorination, and pesticide contamination were excluded as possible causes of the outbreak.

Microcystins are cyclic peptides that are potent liver toxins. They are produced by several genera of cyanobacteria and have been detected in water worldwide, particularly in Brazil.²¹ Hepatotoxicity of microcystins occurs in domestic and wild animals. Animals die within hours to days after the initial exposure, often as a result of intrahepatic hemorrhage and hypovolemic shock. Microcystins cause liver injury through damage to the DNA by the activation of endonucleases.²² The liver accumulates these toxins preferentially and is their chief target organ; the liver rapidly removes microcystins from the blood, but at potentially lethal doses clearance is reduced.²³

In laboratory animals, the concentration of microcystins in the liver that is associated with acute hemorrhagic shock syndrome is approximately 60 to 70 μg per kilogram of body weight (intraperitoneal median lethal dose).²⁴ In animals that survive the initial shock syndrome, hepatic insufficiency may develop to a degree that causes death. The concentra-

tion of microcystins detected in the liver tissue of the case patients who died was similar to the concentrations associated with severe liver damage in animals. Furthermore, the pathological features of the liver tissue of the case patients were similar to those in animals (i.e., disruption of liver plates and other cell deformities).²⁴

In humans, ingestion of toxins produced by cyanobacteria has been reported to cause epidemic gastroenteritis.²⁵ Dermal exposure has caused skin and eye irritation, rhinorrhea, dizziness, and fatigue.²⁶ We are not aware of reports of parenteral exposure to any microcystins among humans.

Our investigation had several limitations. Medical records at the dialysis center and water-treatment records at the city's water plant were poor. Water samples from the time of probable exposure were unavailable, so we could not quantitate exposure. We were unable to obtain serum from patients who received dialysis in Caruaru and were exposed to treated water only.

Cyanobacteria are widespread in natural water sources worldwide. This report of human parenteral exposure to microcystins demonstrates the importance of proper municipal water treatment and appropriate design, installation, monitoring, and maintenance of water-treatment systems in hemodialysis centers in preventing potential exposure to microcystins.

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