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EPIDEMIC DENGUE 4 IN THE YUCATÁN, MÉXICO, 1984.

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SUMMARY

An outbreak of dengue 4 occurred in the Yucatán, México in 1984. During the course of the outbreak, 538 of 5486 reported cases of dengue-like illness were studied; 200 were confirmed as dengue serologically and/or virologically. Dengue 4 virus was isolated from 34 patients and dengue 1 from one. Severe haemorrhagic symptoms were observed in 9 laboratory confirmed patients, including four deaths. Thus, the outbreak in Yucatán is the second dengue epidemic in the Americas after the Cuban epidemic in 1981 in which a number of patients suffered from haemorrhagic complications. It was notable that 5 of 9 hospitalized, severe cases were young adults and that only one met the WHO criteria of DHF, in contrast to primary pediatric nature of DHF in Southeast Asia. In this paper we describe clinical, serologic, and virologic studies conducted during the outbreak.

KEYWORDS: Dengue 4, Outbreak; clinical signs and symptoms; Haemorrhagic cases.

INTRODUCTION

Little information is available on the occurrence of dengue-like illness in the American region between 1780 and the late 1820's [4]. From that time on, however, epidemics have occurred in the region at irregular intervals.

México participated successfully in the *Aedes aegypti* eradication program in the 1960's, and, as a consequence, had no reported dengue transmission until early 1970's. Reinfestation of the country by *Ae. aegyti* occurred sometime in the 1970's, and the first cases of dengue in México were reported from the southeast state of Chiapas in 1978. By the following year, dengue transmission was reported in 8 states, and over the next five years outbreaks were reported in the majority of the states of México [17]. Dengue 1 was the only virus documented in México until 1982 and 1983 when the presence of dengue 2 and dengue 4 respectively, was confirmed. Since 1984, simultaneous transmission of three serotypes (dengue 1, 2 and 4) has occurred in México.

In the Yucatán, the first cases of dengue-like illness were reported in 1979, and cases have been reported every year since that time [1]. In the spring of 1980, dengue 1 was isolated from a patient in Mérida [2]. In 1984, an outbreak of dengue 4 associated with severe haemorrhagic disease occurred in Yucatán with 5,486 cases of dengue fever reported in the state. Here we describe the clinical, serologic and virologic studies on

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538 patients observed from November, 1983 to December, 1984. Clinical and laboratory data on the patients who had confirmed dengue with severe hemorrhages during the epidemic of dengue 4 are also reported.

MATERIALS AND METHODS

Patients

Blood samples were taken from 538 patients with suspected dengue fever who submitted to the Regional Research Center, Universidad Autónoma de Yucatán, a variety of clinics at State institutions of health, or to private clinics. Most of the patients were residents of Mérida City, but some of them were from rural areas nearby Mérida.

Clinical Studies

At the time the first (acute) blood samples were taken, a clinical/epidemiologic questionnaire was filled out for each patient. For hospitalized patients, clinical information was obtained by reviewing the hospital records. All patients were instructed to return in 7-10 days for a second blood sample. Clinical laboratory tests (WBC, Haematocrit (HT), platelet count and urine analysis) were done at the Regional Research Center or at the hospital laboratory.

Serologic and Virologic Studies

Each serum was initially screened for Flavivirus antibody by the hemagglutination-inhibition (HI) test using 4-8 units of dengue 1 antigen [3]. All serum specimens were subsequently tested for HI antibody to the four dengue serotypes, yellow fever, St. Louis and eastern equine encephalitis viruses. Some serum samples were also tested by the complement fixation (CF) test [22] and for IgM antibody by the IgM-capture ELISA [13].

Serologic confirmation of dengue infection was considered if a four-fold or greater rise in HI or CF antibody titer to one dengue serotype occurred between acute and convalescent serum samples taken in the first five days after the onset of illness and 7-10 days after the first bleeding, respectively. Primary dengue infections were confirmed, if the antibody titer of the convalescent serum sample was $\leq$1:640 [5]. Patients were considered to have a presumptive secondary dengue infection, if they had an HI antibody titer of 1:1280 or greater in either sample. Virus isolation was by inoculation of serum sample into *Toxorhynchites amboinensis* (TRA-284) cells grown in Leibovitz-15 medium [9, 14] and/or by intrathoracic inoculation adult *Ae. aegypti* or *Toxorhynchites* mosquitoes [11, 18]. Briefly, a 0.05 ml aliquot of the acute phase serum was inoculated into a tube culture of TRA-284 cells. Two ml of maintenance medium was added, and the tube was incubated at 28°C for 10 days. The cells were suspended in the spent medium, and the suspension was centrifuged at 1000 rpm for 10 min. After removing approximately 1 ml of the medium, the pelleted cells were resuspended in the remaining medium and spotted on 2 Teflon coated 12-well slides [9]. The slides were processed for dengue virus infection by the direct fluorescent antibody test (DFA) using flavivirus-reactive anti-dengue human serum conjugated with fluorescein isothiocyanate (FITC) [9]. Viruses were identified by the indirect fluorescent antibody test (IFA) using serotype-specific monoclonal antibodies against the 4 dengue viruses [9]. When virus isolation in mosquitoes was conducted, undiluted acute serum was inoculated into adult *Toxorhynchites amboinensis* or *Ae. aegypti*. After 14 days incubation at 30°C, surviving mosquitoes were killed by freezing and tested for virus infection by DFA test on brain squashes. The bodies of 5 of these infected mosquitoes were used to prepare antigen for identification by the CF test [12].

RESULTS

The first evidence of the outbreak was detected in July, two months after the onset of the rainy season in 1984. Epidemic transmission peaked in September and few cases were detected by November. No cases were reported in December (Fig. 1). Dengue infection was

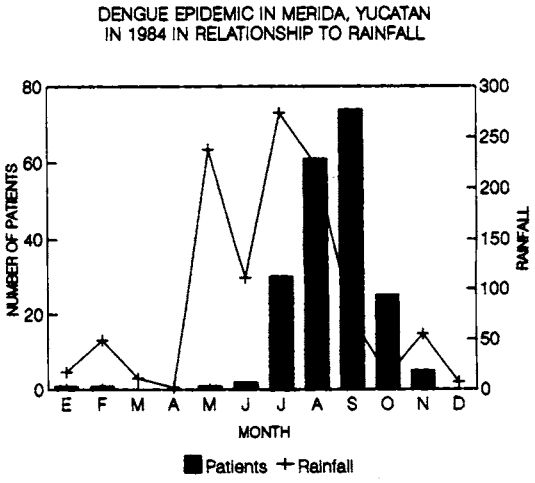

Fig. 1 - Number of dengue fever cases and rainfall by month, Mérida, 1984.

confirmed in 200 out of 538 patients by virologic and/or serologic tests, while 131 were not dengue infections. The etiology of the illness in the remaining 207 patients with single samples could not be determined because neither specific IgM antibody, virus, nor HI titer (≥ 1:1280) could be detected.

Of the 200 patients with confirmed dengue infection, 92 (46%) were male and 108 (54%) were female. All age groups were involved, but the majority of cases were older children and adults (above 10 years old). Only 2 (1%) patients were classified as primary infections, while 189 (94.5%) were secondary infections and nine could not be classified (Table 1).

Table 1

Confirmed dengue cases in Yucatán, by age, sex and serologic response

Age group	Total Nº cases	Sex Male	Female	Serologic Response Primary	Secondary	Not classified
0-10	12	5	7		10	2
11-20	46	19	27		42	4
21-30	37	16	21	1	36	
31-40	45	19	26	1	42	2
41-50	35	18	17		34	1
50+	22	12	10		22	
unknown	3	3			3	
Total	200	92	108	2	189	9

Two serotypes were isolated, dengue 4(34 isolates) and Dengue 1(one isolate).

Most (>80%) patients presented fever, headache, myalgia and retro-orbital pain as the most common symptoms (Table 2). Arthralgia, nausea and chills were also common. Rash was observed in only 63 (31.5%) patients. At least one haemorrhagic manifestation was observed in 45 cases, petechiae and/or a positive tourniquet test being the most common. Melaena and macroscopic haematuria were less common (Table 3). Microscopic haematuria was observed in 26% and proteinuria in 69% of the confirmed dengue cases.

White blood cell counts were recorded in 89 confirmed dengue patients. Counts between 1,000 and 4,900 per µl were observed in 43, between 5,000 - 9,900 per µl in 41 and between 10,000 - 25,000 per µl in 5 patients.

Platelet counts were available from 114 confirmed dengue patients. Thrombocytopenia (platelet count of less than 100,000 per µl) was observed in only 6 patients, while counts between 101,000 - 150,000 per µl were observed in 14 patients. For the rest of the patients the results were within normal limits.

Frequencies of patients with different degrees of severity are broken down by age and sex (Table 4). Both, mild (non-haemorrhagic signs or non hospitalized cases) and severe (hospitalized) was observed in all age groups and both sexes, but those haemorrhagic cases with either one or several haemorrhagic manifestations were more frequent in the female group (Table 4). Nine patients had a more severe haemorrhagic disease, four with a fatal outcome. Death was due to upper gastrointestinal bleeding and shock. Only one case, a nine year old female, met the WHO case definition for dengue haemorrhagic fever [23]. The fatal cases were all females, ages 8, 9, 19 and 37 years. Five survivors included 3 males (ages 5, 11 and 38 years) and 2 females (ages 19 and 43 years). Dengue 4 was isolated from four of the 9 cases with severe haemorrhagic

Table 2

Frequency of nonspecific constitutional signs and symptoms associated with laboratory confirmed dengue infections, Yucatán, 1983-1984

Sign or symptom	Number of cases	%
Fever	199	99
Headache	183	91
Myalgia	166	83
Retro-orbital pain	160	80
Arthralgia	135	67
Nausea	134	67
Chills	131	65
Photophobia	96	48
Pruritus	72	36
Sore throat	68	34
Rash	63	31

Table 3

Frequency of haemorrhagic manifestations associated with dengue infection, Yucatán, 1983-84

Haemorrhagic manifestation	Number of cases	%
Positive tourniquet	56	28
Petechiae	32	16
Epistaxis	10	5
Ecchymosis	9	4
Bleeding gums	7	3
Haematemesis	6	3
Melaena	2	1
Macroscopic haematuria	2	1

Table 4

Correlation of the age and sex with the severity of the illness in laboratory confirmed dengue cases in Yucatán Peninsula, 1984

Age Group	Total Cases	Mild Cases*		Cases with at least one Haemorrhagic manifestation**		Severe Cases***	
		F	M	F	M	F	M
0 - 4	1	1	0	0	0	0	0
5 - 9	11	2	3	2	1	2	1
10 - 14	21	8	8	3	1	0	1
15 - 19	26	10	8	4	2	2	0
20 - 39	81	34	28	12	5	1	1
40 +	57	23	28	3	2	1	0
Unknown	3	0	2	0	1	0	0
Total	200	78	77	24	12	6	3

* No haemorrhagic sign.
** At least one of the following haemorrhagic symptoms: epistaxis, ecchymosis, petechiae, gum bleeding, haematemesis, melaena and/or haematuria.
*** Hospitalized cases.

disease, including one fatal case. The other five were confirmed by serology (Table 5).

Clinical descriptions of five hospitalized cases are described below.

Case 1. A female age 9, from a rural community 64 Km from Mérida. She had sudden onset of fever, chills, vomiting, weakness and malaise. Three days after onset, she had haematemesis and was transferred to a hospital in Mérida. On admission, she was febrile and experiencing upper gastrointestinal (GI) bleeding, had petechiae, ecchymoses, and a hematoma in the right groin. Initial laboratory examination showed WBC count of 13,000/µl, haematocrit 46% and a platelet count of 30,000/µl. On day 4 of illness, she had Hb 13 g/l, Ht 40%, leukocytes 26,000 per µl and platelet 164,000 per µl. The patient was treated with ampicillin, cimetidine, dexamethasone, fluid therapy and blood transfusion. Three days after admission, the patient suffered a cardio-respiratory arrest and died. The HI titer on day 5 of illness was ≥1: 5,120 and by CF test 1:256 against dengue 4 antigen. Diagnosis: presumptive DHF.

Case 2. A female age 19, from a rural town 109 Km from Mérida. She was admitted to a city hospital 4 days after onset of illness. On admission she was febrile with signs of shock. She was confused, drowsy, with mild mydriasis and hepatomegaly, ecchymotic lesions on the arms, legs and abdomen and a history of haematemesis. Laboratory tests revealed a white blood cell (WBC) count of 10,000 per µl, hemoglobin 9.3 g/l, haematocrit 30% and the coagulation time was 15 minutes. The low haematocrit value could be explained because the patient received intravenous fluid therapy. The patient had cardio-respiratory arrest and died 24 hours after admission. The dengue HI antibody titre against dengue 4 antigen in a serum taken on the fifth day of illness was 1:1,280. Dengue specific Ig-M antibody was also detectable in this serum. Diagnosis: presumptive dengue with severe haemorrhagic manifestations.

Case 3. A female age 37, with antecedent chronic peptic ulcer from a rural town 28 Km away from Mérida. A month before onset, she was hospitalized for severe peptic ulcer and upper GI bleeding. Her illness started with fever, malaise, myalgia, arthralgia, abdominal pain, nausea and cough. After three days, she was admitted to a hospital with severe haematemesis and melaena. She was pale, clammy, drowsy, had perioral cyanosis and the fingers were cyanotic. Intravenous fluid therapy was administrated. There was no response to treatment and she died two days later. The dengue HI antibody titer in serum taken on day 5 of illness was 1:320, but the IgM-ELISA was positive. Dengue infection was confirmed by isolation of dengue 4 from the serum. Diagnosis: confirmed dengue with haemorrhagic manifestations.

Case 4. A female age 8, from a community 63 Km from Mérida. She was a deaf-mute with a previous diagnosis of neonatal cerebral damage with psychomotor delay. She was admitted to hospital after 7 days of fever, chills and abdominal and retro-ocular pain. On admission, she was febrile, with a history of haematemesis, melaena, epistaxis and petechiae over the whole body. The haematocrit was 16%, platelet count 50,000 per µl and WBC 25,000 per µl. The next day GI bleeding persisted and was observed by nasogastric

tube. She had epistaxis and bleeding gums. The child died 48 hours after admission due to cardiorespiratory failure. The HI antibody titer serum taken on days 7 and 12 (the day she died) were 1:1280 and 1:5120, respectively. IgM-ELISA test was also positive. Diagnosis: confirmed dengue with haemorrhagic manifestations.

Case 5. Male age 38, from Mérida, presented at the hospital with fever and angina-like pain in the chest and left arm. During the following days he developed pharyngitis, epistaxis, nausea, vomiting, macular rash on face and arms, pruritus, myalgia, arthralgia, headache, photophobia, abdominal pain, petechiae and haematuria. The tourniquet test was positive. WBC count was 5,000 with 78% neutrophils, 6% band forma and 5% lymphocytes. The platelet count was 160,000 and the haematocrit 44%. The HI titers were 1:10 in the first serum and 1:10,240 in a serum taken five days later. Dengue 4 was isolated from the acute phase serum. Recovery was uneventful and complete. Diagnosis: confirmed dengue with haemorrhagic manifestations.

DISCUSSION

Except for a large DHF/DSS outbreak in Cuba in 1981, severe cases of dengue fever had been rarely reported in the American region prior to 1984. Thus, our documentation of 9 hospitalized cases including 4 deaths suggests a beginning of significant change in clinical manifestation of dengue fever in México. Further, the fact that only one patient met the WHO criteria of DHF despite a larger number of severe cases suggests that uniform application of the WHO criteria to the patients of all ages and to the western hemisphere is problematic.

In our study, macroscopic haematuria was observed in 2 patients (1% of all cases). A similar frequency was recorded in Cuba in 1981, where haematuria was seen in three cases of secondary infection (3%) [10]. In the outbreaks in the Philippines in 1956 and Bangkok in 1958, haematuria was rare [20]. On the other hand, in a survey conducted in 1977 in St. Thomas and Puerto Rico in patients eighteen years old or less, haematuria was not found [15]. By contrast, significant haematuria was observed in Tahiti (30%) [16], in Puerto Rico(9%) [19], and in Thailand (80%) [6]. In our study, the routine urinalysis revealed proteinuria in 69% and microscopic haematuria in 26% of patients, which were similar to the observations in Thailand.

Most of the outbreaks caused by dengue 4 virus in the Americas have generally been classical dengue fever. The 1984 experience in Yucatán, however, demonstrates that not all dengue 4 infections are mild and reinforces the fact that all four dengue serotype can cause severe and fatal disease [21].

Six out of 9 severe cases (67%) were female and 4 (44%) died. Still, only one patient (N° 1) met the WHO case definition for DHF [23]. It is worth analyzing why relatively fewer severe cases (including fatality) of dengue in The Americas meet the WHO definition of DHF/DSS. The WHO classification of DHF is based on clinical observations in children in South-East Asia and

Table 5

Haemorrhagic disease cases confirmed dengue infection

Case	Age	Sex	Serology	Virus isolation	Haematocrit %	Platelet count**	Fatality
1	9	F	CF	Neg.	46	30	+
2	19	F	HI, CF, ELISA	Neg.	30	-***	+
3	37	F	ELISA	DEN-4	15,12,23*	100	+
4	8	F	HI,CF,ELISA	Neg.	16	50	+
5	38	M	HI,ELISA	DEN-4	44	160	
6	11	M	HI	-	-	-	
7	43	F	HI,CF,ELISA	Neg.	38	144	
8	5	M	ELISA	DEN-4	-	-	
9	19	F	HI,CF	Neg.	40	137	

* Results obtained on days 3, 4 and 5 after the onset of symptoms.
** x $10^3/\mu l$
*** Not determined

Western Pacific Regions. In contrast, five of the cases in Mérida were adults. This could be one reason why our cases did not meet the clinical requirements of the WHO classification. However, in the Americas, DHF has been previously documented in adult patients [10]. In this study, it was not possible to obtain a reliable haematocrit in some patients because intravenous fluid infusions had been used when the blood samples were taken.

Circulation of multiple serotypes (hyperendemicity) is considered to increase the risk of epidemid DHF. In this study the high fatality rate was due to lack of early diagnosis and proper treatment. In order to prevent the high mortality caused by DHF, health agencies should implement a proactive surveillance system that includes dissemination of pertinent information to the public, training of physicians on clinical diagnosis and treatment, and hospitalization plans for both the cities and the rural communities [8]. Emphasis should also be placed on government programs to improve environmental sanitation and to facilitate community organization and participation in mosquito control programs [7]. Further, prevention and control programs should be based on properly trained personnel and on an adequately equipped laboratory to support clinical diagnosis of the disease.

RESUMEN

Epidemia de dengue 4 en Yucatán, México, 1984.

Un brote de dengue 4 ocurrió en Yucatán, México en 1984. Durante el curso del brote, 538 de 5486 casos reportados como dengue clínico fueron estudiados; 200 fueron confirmados como dengue, por estudios serológicos y/o virológicos. El dengue tipo 4 fue aislado de 34 pacientes y dengue 1 de un paciente. Síntomas hemorrágicos severos fueron observados en 9 pacientes confirmados por pruebas de laboratorio, de los cuales 4 fallecieron. Así, el brote en Yucatán es la segunda epidemia de dengue en las Américas después de la epidemia en Cuba en 1981 por el número de pacientes que sufrieron de complicaciones hemorrágicas. Fué notable que 5 de 9 casos hospitalizados, fueron adultos jóvenes y que únicamete un paciente reunió los criterios de la Organización Mundial de la Salud para la Fiebre Hemorrágica por Dengue (FHD), en contraste con los casos pediátricos de FHD del Sureste de Asia. En este artículo describimos los estudios clínicos, serológicos y virológicos realizados durante el brote.

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