A KILLING DISEASE EPIDEMIC AMONG DISPLACED SUDANESE POPULATION IDENTIFIED AS VISCERAL LEISHMANIASIS

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Abstract. A fatal disease epidemic affected the Bentiu area in southern Sudan and led to a mass migration of the Nuer tribe searching for treatment. The initially available information revealed a high mortality rate due to a possible occurrence of tuberculosis, malaria, enteric fever or visceral leishmaniasis (VL). Serological screening of 53 of the most severely affected patients in an enzyme-linked immunosorbent assay (ELISA) or an improved direct agglutination test (DAT) revealed positivity for VL. In 39 of those patients, diagnosis was confirmed by identification of *Leishmania donovani* amastigotes in lymph node or bone-marrow aspirates. In a total of 2714 patients observed, 1195 (44.0%) had clinical symptoms suggesting VL: DAT positive titers (1:3200-≥ 1:12800) were obtained in 654 (24.1%), of whom 325 were confirmed parasitologically. Forty-two VL cases died before or during treatment, giving a mortality rate of 6.4%. Among the intercurrent infections diagnosed in the VL population (654), respiratory involvements (31.7%) and malaria (10.7%) were most prevalent. With the exception of four (0.6%), all other VL patients (509) responded readily to sodium stibogluconate. The factors initiating the outbreak are discussed. Malnutrition and nomadic movements to potential VL endemic areas appeared to be the most important. HIV infection as a possible predisposition seemed remote considering the clinical and epidemiological similarity to VL occurring in East Africa, adequate humoral response in DAT, and immediate positive response to specific anti-*Leishmania* chemotherapy.

The first case of visceral leishmaniasis (VL) in the Sudan was diagnosed in 1904;[1] thereafter, the endemic area was identified to be along the bank of the Blue Nile and its tributaries (Fig. 1). An outbreak among soldiers stationed in the northern Upper Nile Province was reported thirty-six years later.[2] The most famous outbreak was in the period 1956–1958, involving the region between the Blue and White Nile and Kassala Provinces up to the borders with Ethiopia.[3] Among the tribes residing in this area, the Jum Jum was most affected, having a mortality rate of more than 50%.

In early October of 1988, we received the first information of an outbreak of a killing disease among migrants arriving in Khartoum from the Bentiu area in the Upper Nile Province.[4] The outbreak had led to a mass migration of the Nuer tribe to the northern towns of Kadugli, Kosti, El Obeid and Khartoum seeking treatment. This information indicated that the disease had not only affected the Bentiu area but also the adjacent areas of Ayod, Adok and Waat, and that the mortality rate was extremely high among the members of the Nuer tribe. On the basis of this information it was also apparent that the disease had not originated in Bentiu but was introduced by nomads from the adjacent areas of Ayod and Waat. Considering the severe nature of the disease involved, it was obvious that those who succeeded in reaching Khartoum represented a small proportion of the real number affected in the main focus. The majority of the population was dying along the migration route. The high mortality among those who arrived in Khartoum initiated investigations by our team to identify and control the contagious disease. Hindered by difficulty in reaching the main focus in Bentiu, due to the continuous civil war in the region, our efforts were concentrated on managing the disease in the displaced population in Khartoum. Similar measures were also undertaken for thousands of others from the same tribe settled in towns and villages along the 900- kilometer migration route.

MATERIALS AND METHODS

Identification of the disease

Our first impression was that the killing disease could be tuberculosis, enteric fever, chronic
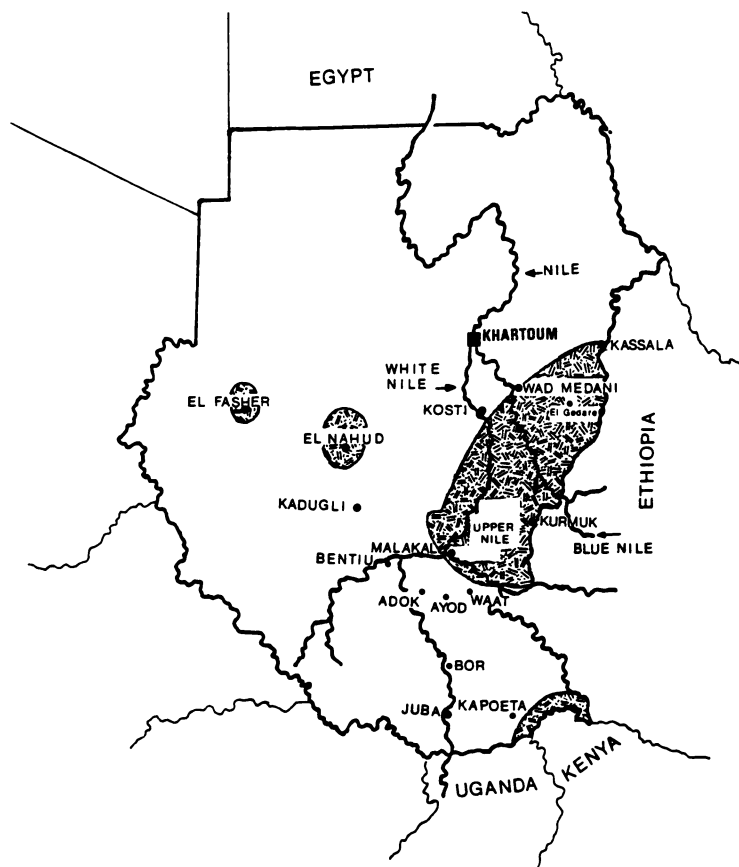
FIGURE 1. Known endemic areas of visceral leishmaniasis in the Sudan. Scale: 1 cm = 120 kilometers.

malaria or visceral leishmaniasis. Since African trypanosomiasis has not been previously reported in Bentiu area and its typical clinical symptoms were not observed in any of the patients examined, it was excluded as a possible infection in this epidemic.

Because of the unknown nature of the pathogen involved, precautions were taken to prevent its spread to the local community in Khartoum. Due to the limited capacities of the hospitals there, patients were visited at their settlements around the city.

In order to identify the etiological agent, blood and sputum samples were collected from the most severely affected patients (53). Repeated blood film examination for malaria parasites, Ziehi-neelsen staining for identification of acid-fast *Mycobacteria*, or the Widal test for detection of antibodies to *Salmonella* sp. were performed.

Blood and serum samples from those patients were also tested in ELISA (10) and DAT (53) for detection of specific anti-*Leishmania* antibodies.[5, 6]

Hospitalization and management

An emergency hospital was erected to comply with the necessary precautions for performing organ aspiration and administration of chemotherapy. In order to cope with the expected pressure, temporary wards were constructed in district hospitals along the migration route to Khartoum.

Before admission to the emergency hospital, all administrative information and case histories of each patient were registered. Clinical and hematological examinations including body weight, temperature, liver and spleen size were regularly performed, and hematological parameters such as hemoglobin concentration, and total and dif-

ferential leukocytic counts were determined when required. At the attached laboratory blood films, organ aspirates, sputum and fecal samples were also examined. The DAT and ELISA for VL were performed at Michigan State Laboratory in Khartoum.

All patients received well-balanced diets to ensure good response to treatment, and multivitamin supplements were administered regularly.

Diagnosis of VL

Out of 2714 persons examined for various clinical disorders, 1195 were suspected for VL. Prevalence of VL was also assessed in Nuer migrants from the same area (Bentiu) who had settled in Khartoum 6–12 months earlier; blood specimens were collected from 343 persons randomly selected from two groups. As controls for DAT performance, the following serum samples were included: 18 healthy individuals (10 Dutch and 8 Sudanese); 20 parasitologically confirmed VL cases (10 Kenyans and 10 Sudanese); 45 Sudanese patients with confirmed malaria (15), tuberculosis (18) or schistosomiasis (12).

Patients with clinical suspicion of VL and positive DAT titers (≥1:3200) were subjected to parasitological confirmation. Giemsa-stained aspirates from enlarged lymph nodes (inguinal or cervical glands) or bone-marrow (iliac crest) were judged for presence of amastigotes by at least two trained persons. Since we were not able to guarantee the required safety measures in our provisory hospital, spleen and liver punctures were not performed. The hemorrhagic signs and epistaxis were additional contraindications. Taking into account the size of the population to be examined and the number of manipulations required for its performance, bone-marrow aspiration was only done at the initial phase of our investigations for confirmation of VL.

Patients suspected for VL were also assessed for intercurrent infections such as tuberculosis, malaria and other endemic diseases. When diagnosed, patients with intercurrent infections were treated simultaneously or after completion of therapy against VL.

Justification of treatment

After confirmation of VL by lymph node aspiration, patients were put immediately on anti-Leishmania chemotherapy. Negative parasito-

logical findings did not exclude suspicion for VL since this method is known to be insensitive. Culturing of aspirates, which might have had improved sensitivity, was not manageable due to the frequent microbial contamination, irregular electric current for optimal incubation and the very high laboratory temperature (30–35°C).

Delay in administration of anti-Leishmania chemotherapy was considered risky as patients were in extremely poor condition. Due to a shortage of ELISA antigen and lack of facilities to perform other serological tests, administration of chemotherapy in the unconfirmed cases was mainly based on clinical symptoms typifying VL, and DAT-positive results. Reliance upon DAT positivity was justified by the required sensitivity and specificity experienced in 15 different endemic areas of VL.[6-10]

Treatment procedures

Sodium stibogluconate (Pentostam) is considered the most effective drug against East african VL.[11] The full dose schedule recommended by WHO Expert Committee was followed: 10mg/Kg body weight iv was prescribed for adults. Children up to 12 years of age received daily doses of 200–400 mg respectively.[12]

Before subjection to full course treatment, patients were evaluated for positive responses in a 5-day therapeutic test starting with one-sixth of the recommended dose, thereafter increasing gradually to reach the full dosage on day 6. Initial positive responses such as disappearance of fever, physical improvement and absence of side effects, justified full course treatment for 30 days. At the time of this report, 513 out of 663 diagnosed VL cases were treated according to the regimen described above. The criteria considered for successful treatment and subsequent discharge from the hospital were: disappearance of fever, regression in spleen size, weight gain, cessation of hemorrhagic diathesis and improvements in general condition. Considering the large number of VL cases, the hematological responses were evaluated in only a few patients (30).

RESULTS

Of the first group of 500 patients examined, the vast majority (98.9%) were from the Nuer tribe. The incidence of the disease was slightly higher in males (57%) and all age groups were

TABLE 1

Distribution of clinical symptoms observed in the first
500 patients examined

Clinical symptom	Number of patients	
Fever	500	100%
Splenomegaly	419	83.8%
Anemia	164	32.8%
Hepatomegaly	158	31.6%
Emaciation	102	20.4%
Epistaxis	37	7.4%
Lymphadenopathy	30	6.0%
Hyperpigmentation	2	0.4%

TABLE 3

Intercurrent infections identified in 654 VL patients

Clinical condition	Number of patients	
Upper respiratory tract involvements	136	20.8%
Pneumonia	67	10.2%
Tuberculosis	4	0.6%
Diarrhea	120	18.3%
Malaria	70	10.7%
Giardiasis	17	2.6%
Amoebiasis	15	2.3%
Mucocutaneous Leishmaniasis	7	1.1%

affected: 40% were children younger than 15, 40% in the 15–34 years of age group and 20% were older than 35. The prevailing symptoms are presented in Table 1.

The absence of tuberculosis, malaria, enteric fever and African trypanosomiasis in the 53 most severely affected patients indicated a higher probability of VL. In 10 samples from this group screened by both ELISA and DAT and in 43 screened by DAT only, results indicated VL (absorbances ≥ 0.5 and titers ≥ 1:3200). The seropositivity to VL obtained by DAT and ELISA in these 53 patients indicated that VL was most likely to be the major "killing disease" in this epidemic. Leishmania donovani amastigotes were demonstrated later by lymph node (29) or bone-marrow (10) aspiration in 39 of them. Although in further diagnosis special emphasis was placed on VL, the possible presence of other infections was not excluded.

Of 2714 patients examined, 1195 (44.0%) had clinical symptoms of VL. DAT-positive readings (titers ≥ 1:3200) were obtained in 654 (24.1%); L. donovani amastigotes were demonstrated in 325 of them. Titers ranging from 1:800–1:1600 were obtained in 38 patients with no obvious symptoms of VL (Table 2). Forty-two confirmed

VL cases died before or during treatment, giving a mortality rate of 6.4%.

In the seroprevalence survey carried out in the randomly selected group (Table 2), nine individuals (2.6%) scored DAT titers indicative of VL. Two of these nine were shortly afterwards diagnosed by lymph node as VL cases. In the remaining seven no VL symptoms were observed; they were considered for a follow-up study not yet completed.

Among the intercurrent infections diagnosed in the VL population, respiratory tract involvements, diarrhea and malaria appeared to be the most dominant (Table 3). Epistaxis was one of the serious problems encountered in VL management; 1–3 cases were seen daily. Its peak was during the three hottest months of April, May and June. Application of adrenalin-impregnated gauze appeared to be effective in most of the patients. In persistent cases, subjecting VL patients to a cooler air current from a fan proved to be helpful. Examination of blood smears from those patients showed no Borrelia infection as was first anticipated. As an additional precaution, acetylsalicylic acid was banned from the hospital.

TABLE 2

DAT titer distribution for VL in clinically suspected and randomly selected populations from Bentiu area

Diagnosis	Total number tested	DAT titer range*			
		≤1:400	1:800–1:1,600	1:3,200–1:6,400	≥1:12,800
Clinically suspected for VL**	1195	503	38	59	595
Randomly selected population***	343	328	6	0	9
Control group with other infections	45	44	1	0	0
Confirmed VL control group	20	0	0	0	20
Healthy individual control group	18	18	0	0	0

* DAT cut-off titer = 1:3,200.
** Patients from the affected population Nuer tribe under study.
*** Individuals from the same tribe settled 6–12 months earlier in Khartoum.

Almost all (509/513–99.4%) VL cases treated showed immediate positive response to Pentostam administration. Fever subsided during the first week and an average decrease of 4.4 cm in spleen size was observed during the course of treatment. At the time of admission to the hospital, the average total leukocyte count was 2500 cells/mm³ and at the end of treatment this ranged from 4000–12900 cells/mm³. There was cessation of epistaxis and hemorrhagic diathesis and improvements in the general condition of the treated patients. Primary unresponsivness was seen in two VL cases; after a second course of Pentostam they were cured. Two others did not show improvements, even after a second course of treatment; they were referred to the Academic Hospital in Khartoum.

No major toxic side effects were observed during the course of treatment with Pentostam. Six VL patients with cardiac dysfunction due to pericarditis or anemic heart failure were treated without sequelae.

DISCUSSION

The factors that initiated this severe VL outbreak are not yet fully understood. On the basis of available information, VL was not among the important endemic infections in this area. The last devastating VL outbreak in the Sudan was reported 32 years earlier in the Fung area, hundreds of kilometers east of Bentiu. However, the affected population (Nuer tribe) are nomadic cattle owners. Their seasonal migration to the northern part of this province or the southern borders of the Blue Nile Province may have resulted in importation of the disease. Migration movements from VL-endemic areas appear to be most important in previous outbreaks that had occurred in the Sudan. Of a similar magnitude were those reported in the southern Fung and Melut areas, where VL was imported by nomadic Arabs of Ruffa El Hoi from Singa and patrolling soldiers from Jongols post.[2, 3]

The continuous civil war has destabilized the agricultural activities, nomadic movements and health services in this region. Likewise, transportation of food from the north and implementation of health care measures including control of malaria and VL were interrupted. The implication of those deficiencies was clearly reflected in the severe malnutrition signs in the population studied. The combination of these factors may

have also contributed in this VL outbreak. Previous studies have shown that VL can be latent for very long periods, acquiring an overt clinical form after exposure to stresses such as malnutrition.[13, 14] To the best of our knowledge, serious intercurrent infections other than malaria, respiratory tract involvement or tuberculosis, were not identified. In contrast to other studies where HIV infection or auto-immune disorders occurred concurrently with VL,[15] all of our VL patients with or without intercurrent infections responded well to chemotherapy and proper nourishment.

The positive DAT titers obtained in all VL patients so tested exclude the possibility of immunodeficiency as far as the humoral response is concerned. In previous studies carried out in southern France,[7] two out of four patients with positive HIV tests and confirmed VL showed clearly negative DAT titers. The positivity of DAT in the other two was attributed to an early stage of HIV infection at which the humoral response is known to be still functional. For ethical and social reasons we were not able to subject some of our patients to an HIV test.

Since our efforts to reach Bentiu were not successful, the role played by the sand fly or that of the animal reservoir in this VL outbreak remains undetermined. Phlebotomus orientalis is the major vector for leishmaniasis in the south of Sudan, and considering the favorable ecology of the area, this species can be present in large numbers.[16] Depending on the availability of the etilogic agent, L. donovani, and the prevailing climatological conditions, up to 4% of the population can be infected.[17]

Unlike the Mediterranean littoral where the dog is known to be the major source of VL, rodents such as Rattus rattus and Avricanthis niloticus and carnivores including the cat and genet have been incriminated as sources of VL in southern Sudan.[17] Perhaps for reasons related to the better standard of living, the very high incidence of leishmaniasis among the local dog population in the Mediterranean does not correlate with that of VL in the human population. Lowered immune responses due to malnutrition in this affected population may have contributed to an increased susceptibility to L. donovani transmitted from the local animal reservoirs.

In spite of the severe nature of VL in this outbreak, its epidemiological and clinical features were not very much different from those reported

in East Africa.[11, 18] Males (57%) and children below 15 years of age (40%) were most affected. The prevailing symptoms were also similar to those reported in an earlier VL outbreak in the adjacent Blue Nile province and in endemic areas of Kenya.[3, 19] Epistaxis and hemorrhagic signs, however, were common in our VL patients, possibly due to the very high ambient temperatures. Reliance on these symptoms did not guarantee differential diagnosis with respect to other commonly occurring diseases in the Sudan.[3] In our judgement, failure to properly diagnose VL at its onset in Bentiu accounted for this high mortality.

In this epidemic the DAT was of great assistance in identification of VL. Its further use in screening procedures provided the required reliability and practicability under the adverse conditions experienced in Sudan. All patients who had positive DAT titers ($\geq$ 1:3200) were found to be either genuine cases of VL or favorably responding to specific anti-*Leishmania* chemotherapy. While in 51.0% of the VL patients, no *L. donovani* amastigotes could be demonstrated, DAT titers ranged from 1:3200– $\geq$ 1:12800. Administration of Pentostam in 509 (99.2%) out of 513 hospitalized resulted in successful treatment. The required specificity of DAT with respect to infections clinically undistinguishable from VL is evidenced in this as well as earlier studies undertaken in Kenya, Brazil and Sudan.[6, 9, 10] Having experienced the devastating effects on this displaced population, we conclude that regular epidemiologic and surveillance procedures are imperative in order to overcome future VL outbreaks.

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