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Infection rates with *Leishmania donovani* and *Mycobacterium tuberculosis* in a village in eastern Sudan

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Summary

BACKGROUND *Leishmania-tuberculosis* co-infection is not uncommon in clinical practice in East Africa, but little is known about the epidemiology of this problem at population level. A cross-sectional household survey was carried out in an active visceral leishmaniasis (VL) focus in Eastern Sudan in February 2002.

METHODS All inhabitants of Marbata village in Atbara River Area, Gedarif State, who gave informed consent, underwent both a leishmanin skin test (LST) and a tuberculin test for infection with *L. donovani* and *Mycobacterium tuberculosis*. All subjects were clinically screened for VL and tuberculosis (TB).

RESULTS About 66% (252 of 382) were LST-positive, 26% (100 of 382) were tuberculin-positive and 20% (77 of 382) were positive for both tests. By the age of 15, more than 60% of inhabitants were LST-positive, but <20% were tuberculin-positive. By the age of 30, these percentages increased to 100 and 50%. No association was found at the individual level between leishmanial and tuberculous infection after controlling for age.

CONCLUSION In this community study, we found no association between the risk of infection with *L. donovani* and *M. tuberculosis*. However, the progression to active VL disease might be different in *M. tuberculosis*-infected than in non-infected persons and vice versa. Prospective studies are needed to document the prognosis of TB/VL co-infection.

keywords visceral leishmaniasis, epidemiology, leishmanin skin test, tuberculin, survey, Sudan

Introduction

Both visceral leishmaniasis (VL) and tuberculosis (TB) are increasing in the Sudan, particularly in the eastern region of the country. While the relationship between HIV and TB (McCarthy *et al.* 1995) has been documented in Sudan, little is known about TB/*Leishmania* co-infection, a syndrome that has important clinical implications. Although distinct in aetiology and transmission mechanisms, VL and TB share several features. Many infections remain asymptomatic. Symptoms usually develop after several months or years in those who progress to clinical disease; very long incubation periods (latent infection) may be related to immune suppression occurring at a later age, which apparently turns the latent infection into active disease.

It has been suggested that TB represents one of the immunosuppressive conditions that can cause the progression of latent leishmanial infection to clinical leishmaniasis (Montalban & Callejia 1990). Similarly, VL can reactivate a latent mycobacterial infection (Chaudhuri 1989).

VL or kala-azar is a disease of public health importance in Sudan, which is considered to be one of the main foci of VL in the world (Desjeux 1996). The disease spreads over a wide belt from the Atbara river in the north-east, along the Sudanese-Ethiopian border to the south of the Sobat river reaching Nasir and Malakal, and extending westwards across the White Nile (Zijlstra & el-Hassan 2001). Recent kala-azar epidemics have devastated the population of southern Sudan in the Bentiu area (Seaman *et al.* 1996) and have severely affected the eastern region in Gedarif State, particularly in the Atbara River area (el-Safi *et al.* 2002). It has been estimated that at least 1000 cases of VL occur each year in Gedarif State (el-Hassan *et al.* 1995). The

annual incidence rate is about 38/1000 person-years and the ratio of clinical to subclinical cases was reported as 1.6:1 by Zijlstra *et al.* (1994).

TB is a major cause of morbidity and mortality worldwide. It threatens one-third of the world's population and is the most common opportunistic infection in HIV/AIDS. The burden of TB is greatest in low-income countries (Raviglione *et al.* 1995) where the disease is exacerbated by the HIV/AIDS epidemic (Narain *et al.* 1992; Lucas *et al.* 1993). TB is a major health problem in Sudan, which, like other African countries (De Cock & Wilkinson 1995; Cantwell & Binkin 1996), is subject to the dual epidemics of TB and HIV/AIDS. The annual risk of TB infection is estimated at 1.8%, which gives an incidence rate of TB smear-positive cases of 90/100 000 person-years, putting Sudan among the high prevalence countries for TB in the Eastern Mediterranean Region (el-Sony *et al.* 2002). Age-specific rates were highest among the age group 25–34 with male predominance (National TB Programme 2003, unpublished document). Pulmonary cases represent 80.6% of TB and extra-pulmonary disease constitutes 19.4%. The National TB Programme has successfully introduced the directly observed treatment short course (DOTS) strategy since 1995 (el-Sony *et al.* 2003).

A number of clinical cases of concomitant VL-TB have been reported in Sudan (Sati 1942; el-Safi *et al.* 1995; Khalil *et al.* 1998) and in other parts of the world (Bryceson *et al.* 1985). However, little is known about the degree of interaction between both infections at community level. We conducted a cross-sectional survey in a Sudanese VL focus to explore age-specific prevalence of TB and VL infection, as well as the extent of co-infection at the community level.

Material and methods

Study area and study population

The survey was organized in Marbata village, located about 100 km south-east of Gedarif town along the Atbara River, in Gedarif State, eastern Sudan, a well-known focus of VL (Hoogstraal & Heyneman 1969). The epidemiology of VL in the area was previously described by el-Safi *et al.* (2002). There are 112 households in Marbata village and the total population is approximately 500. They are predominantly of ethnic Fur origin, and migrated to this area from the western Darfur region in 1917. The villagers work mainly as agricultural labourers and their staple diet is sorghum or millet porridge (*asida*). The extended family lives in a compound consisting of several thatched grass huts and enclosed within a thatched grass fence. Goats, sheep, dogs and donkeys are kept inside the compound. *Acacia* and *Balanites aegyptica* trees grow within the compounds and in the narrow streets. Termite hills are found in the compounds as well as in the surrounding rain-irrigated farms. The village is virtually isolated during the rainy season when roads become inaccessible. There is one primary school in Marbata, but no health facilities, and the nearest dispensary is located in Wad Kawli village, at a distance of about 5 km.

Ethical considerations

The research team first visited Marbata village in April 2001, after approval for the study was given by the federal and state health authorities and ethical clearance was obtained from the Institutional Review Board of the Faculty of Medicine at Khartoum University. Informed consent for the research was obtained from the village People's Committee and the local community leader (Sheikh), as well as from all individuals who participated in the study. Confirmed cases of VL as well as TB were cared for according to the standards of the Ministry of Health.

Cross-sectional survey

Marbata village was mapped and an exact census was made of its population in 2001. A house-to-house survey was carried out in the village in February 2002. Each inhabitant was clinically screened for VL and TB, and his or her demographic and clinical information as well as past history of VL and prior BCG vaccination were recorded on a standard form. Each participant had both the tuberculin and leishmanin skin test (LST), and all patients with clinical features suggestive of VL also underwent lymph gland aspiration.

Laboratory tests

For the tuberculin test, 0.1 μl of 5U-strength purified protein derivative (PPD) (VACSERA, Cairo, Egypt) was intradermally injected according to the Mantoux technique on the dorsal surface of the left forearm. The reaction was read between 48 and 72 h after administration. The transverse diameter of palpable induration (not erythema) was recorded in mm (Sokal 1975). Palpable induration of ≥10 mm at the injection site was taken as evidence of tuberculous infection (Rieder 1995).

For the LST, 0.1 μl of *L. infantum* antigen, supplied by Dr M. Gramiccia, Instituto Superiore di Sanita, Rome, Italy, was injected intradermally on the volar surface of the forearm. The reaction was read after a minimum of 48 h and a maximum of 72 h. We considered a person to be

infected with *L. donovani* if the LST gave a palpable induration of ≥5 mm.

Clinical case definitions

The clinical case definition of a suspected active kala-azar case was somebody with fever for more than 2 weeks and splenomegaly or lymphadenopathy. We searched for post-kala-azar dermal leishmaniasis (PKDL) signs, following the classification of el-Hassan *et al.* (1992).

Data analysis

All data were entered into an EPI INFO V.6 format, and further analysed with STATA. To study associations between nominal variables, we computed relative risks and the Pearson chi-square test. Age was categorized into two groups using the median as cut-off. We used stratified analysis to check for possible interaction or confounding of associations.

Results

A total of 402 of the 500 residents of Marbata village agreed to participate in the study, but only 382 were included in this analysis as tuberculin and/or LST data were missing for 20 persons. The main characteristics of the 382 enrolled individuals are shown in Table 1. About 50% of the study subjects were male, with a mean age of 22.4 (SD: 20.9, range from 0 to 90 years).

About 66% (252 of 382) of the study population were LST-positive, 26% (100 of 382) were tuberculin-positive and 20% (77 of 382) were positive for both tests. Figure 1 shows the distribution of the LST and tuberculin reaction by age group.

The diameter of the positive LST reaction ranged between 5 and 48 mm (mean = 17.8, SD: 6.3) and it increased significantly with age ($P < 0.0001$). About 82% (90 of 110) of the individuals who reported a prior history of VL disease had a positive LST compared with 59% (159 of 269) in the group without prior history of the disease, a significant difference ($P < 0.0001$). However, LST positivity was not significantly different in those with (66.2%) and those without (62.2%) family history of the disease ($P = 0.50$).

About 56% of the 100 tuberculin test-positive villagers were male, and their age ranged from 2 to 90 years (mean = 33.6, SD: 20.6). The diameter of positive tuberculin reaction ranged between 10 and 44 mm (mean = 17.6 ± 6.7), increasing significantly with age ($P < 0.0001$). Tuberculin test reaction and prior history of BCG vaccination were not significantly related ($P = 0.89$).

The LST and tuberculin positivity were weakly associated; the crude risk ratio for TB infection in LST-positive people was 1.73 (95% CI: 1.14–2.62) compared with LST-negatives. However, in the stratified analysis this association between LST and tuberculin positivity disappeared after controlling for age (Table 2).

Table 3 shows the results of the clinical assessment of the study population. Malaria was confirmed by thick film in six of the 35 complaining with fever. Onchocerciasis was clinically suspected in seven persons. Seven persons corresponded to our clinical case definition of a VL suspect (fever for ≥2 weeks with splenomegaly and/or enlarged lymph nodes), but subsequent lymph gland aspiration could confirm the disease in only one of them. This single kala-azar case tested negative in both LST and tuberculin test. Four of the six non-confirmed VL suspects were LST- and tuberculin-positive, one was LST-positive/tuberculin-negative, and another one was negative in both tests.

Seven PKDL cases were detected, five male and two female, with age ranging between 5 and 13 years. The LST was positive in six (85.7%) and none reacted positively to tuberculin. Among the village residents we found 12 active cases of TB who were undergoing DOTS treatment, but all 12 refused enrolment in the study.

Table 1 The main characteristics of subjects included in survey of Marbata village, Gedarif State, Sudan, February 2002

Variables	$n = 382$ (%)
Age ≤13 years	195 (51.1)
Male sex	189 (49.5)
LST-positive	252 (66.0)
Tuberculin-positive	100 (26.2)
Prior history of BCG ($n = 348$)	62 (17.8)
Past history of VL ($n = 379$)	110 (29.0)
Family history of VL ($n = 375$)	293 (78.1)

LST, leishmanin skin test; VL, visceral leishmaniasis.

Discussion

Our study showed very high leishmanial infection rates in Marbata village, and a substantial proportion of the LST-positive inhabitants were also tuberculin-positive. The dynamics of transmission of both diseases are quite different in this community: by the age of 15, more than 60% of the inhabitants were LST-positive, whereas <20% were tuberculin-positive at the same age. After the age of 30 years, almost all of the inhabitants were LST-positive, but only about 50% tested positive for *Mycobacterium tuberculosis* infection.

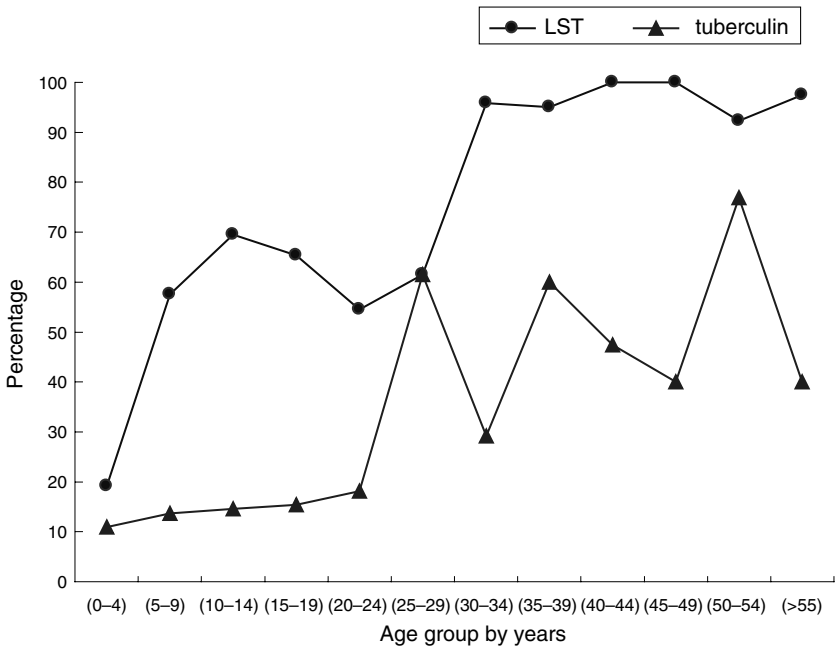

Figure 1 Distribution of leishmanin skin test (LST) and tuberculin positivity by age group in Marbata village, Gedarif State, Sudan ($n = 382$).

Table 2 Association between *Leishmania donovani* and *Mycobacterium tuberculosis* infections, stratified by age, sex and prior history of BCG

	Tuberculin-positive in LST-positive, *n* (%)	Tuberculin-positive in LST-negative, *n* (%)	RR	95% CI
Overall, crude RR	77/252 (30.6)	23/130 (17.7)	1.73	1.14–2.62
Age <13 years	10/90 (11.1)	15/105 (14.3)	0.78	0.37–1.64
Age ≥13 years	67/162 (41.4)	8/25 (32.0)	1.29	0.71–2.36
Summary RR*			1.04	0.65–1.65
Male	44/123 (35.8)	12/66 (18.2)	1.97	1.12–3.46
Female	33/129 (25.6)	11/64 (17.2)	1.49	0.81–2.75
Summary RR*			1.74	1.15–2.63
BCG-positive†	7/26 (26.9)	8/36 (22.2)	1.21	0.50–2.92
BCG-negative	62/203 (30.5)	13/83 (15.7)	1.95	1.14–3.3
Summary RR*			1.72	1.11–2.78

RR, relative risk; LST, leishmanin skin test; CI, confidence interval.
* No evidence for presence of interaction (test for heterogeneity of stratum-specific estimates, $P > 0.05$).
† Prior history of BCG not known for 34 individuals.

This is the first study in Sudan to investigate any relationship between TB and VL infection at the community level. LST and tuberculin tests were previously evaluated only on patients with active kala-azar (Zijlstra & el-Hassan 1993). Both tests have their known limitations. The LST measures delayed-type hypersensitivity and correlates well with the *in vitro* proliferation of mononuclear cells cultured in the presence of leishmanial extracts (Tremonti & Walton 1970). LST becomes positive as *Leishmania* reactive T cells develop and circulate in the blood. However, the significance of a positive LST test for the individual is not entirely clear; it indicates that the person has developed cell-mediated immunity to *Leishmania* but whether this indicates protection against new infection is uncertain. The reaction to tuberculin in a previously tuberculin-positive subject appears to be suppressed when this person develops active VL. However, the tuberculin reaction is restored after successful VL treatment, indicating that immunosuppression in VL is both specific and non-specific (Ho *et al.* 1983; Zijlstra & el-Hassan 1993). For epidemiological work, the LST can be used as an indicator of past infection in the population, although it should be used with caution in populations with high BCG coverage. Rab and Evans (1994) showed

Table 3 Symptoms and clinical signs presented by study population during clinical screening of Marbata village ($n = 382$)

	n (%)
Fever	35 (9.2)
Cough	13 (3.4)
Weight loss	3 (0.8)
Enlarged lymph nodes	102 (26.7)
Splenomegaly	33 (8.6)
Hepatomegaly	22 (5.8)
Clinical suspect for VL	7 (1.8)
Confirmed VL	1 (0.3)
PKDL	7 (1.8)

VL, visceral leishmaniasis; PKDL, post-kala-azar dermal leishmaniasis.

that prior BCG vaccination inhibits the LST reaction. In our study, only 26.6% of the children below 15 years ($n = 188$) had a prior history of BCG vaccination. Therefore, suppression of leishmanin reaction because of prior BCG can only marginally have affected the figure of leishmanin positivity rate in this survey, in the sense of a slight underestimation of the true rate.

Similarly, reactivity to tuberculin does not always mean that the person has been previously infected by *M. tuberculosis*. Because tuberculin contains antigens that are shared with non-tuberculous environmental mycobacteria, sensitization with these organisms can yield a positive skin test in the absence of tuberculous infection. However, Rieder (1995) concluded that in high prevalence countries, the tuberculin test can provide useful information to document tuberculous infection rates at population level. Prior BCG vaccination can induce cross-reactivity with tuberculin PPD, but only a limited number of children in the sample had benefited from prior BCG.

About 66% of the Marbata inhabitants that were included in the present study were found positive on LST and 26% on the tuberculin test. The age-related profile of LST positivity observed in the present study is in agreement with previous reports from Atbara River (el-Safi *et al.* 2002) and Rahad River Areas (Zijlstra *et al.* 1994) in eastern Sudan and other VL endemic areas in the world including Ethiopia (Ali & Ashford 1993), Kenya (Leeuwenburg *et al.* 1983; Schaefer *et al.* 1994), the Mediterranean region (Pampiglione *et al.* 1975; Bettini *et al.* 1977; Gramiccia *et al.* 1990; Marty *et al.* 1992), India (Nandy *et al.* 1987) and Pakistan (Rab & Evans 1994). This pattern of distribution reflects an endemic situation with immunity building up in adolescence and is due to the slow development of *Leishmania* reactive T cells following exposure to infection. However, a different pattern was observed in the epidemiological study conducted by Shiddo *et al.* (1995) in Somalia, who observed a decrease in the number of positive individuals after the age of 39. Accordingly they suggested that VL in that area was accompanied by time-limited cell-mediated immunity.

So far few epidemiological studies have been carried out in Sudan to document the extent of tuberculous infection (WHO 1958; Omer *et al.* 1979). A tuberculin survey amongst Sudanese refugees in Uganda indicated a prevalence of 12% (Migliori *et al.* 1992), whereas we found 26%. The age distribution for tuberculin in the study was significantly associated with age, as expected. This is in accordance with reports from Somalia where Shiddo *et al.* (1995) found a continuous increase in the prevalence of tuberculin-positive reactions with increasing age, indicating lifelong cell-mediated immunity in TB.

No association was found at the individual level between the risk for leishmanial and tuberculous infection after controlling for age. This is hardly surprising, as the exposure pattern and transmission routes are different for both infections. However, our data do not imply that there is no interaction at all between TB and VL. Clinical history of both syndromes is determined by the quality of the cell-mediated immune response; therefore, the prognosis of co-infected individuals might well be different from those of persons coping with single infections. In view of this, it is striking that 12 of the 500 villagers (2.4%) were currently undergoing treatment for smear positive TB, which equals a very high TB prevalence, if we assume them to be all true cases. Unfortunately, all refused enrolment, a major weakness of this study, as we were unable to screen them for VL co-infection. TB still carries an important social stigma in this community, and we suspect this was the reason for their refusal.

We conclude that tuberculous infection rates can be high in foci of active VL transmission in Sudan. No association between the risk of both infections was demonstrated at the individual level, but the progression to active disease is likely to be different in those co-infected from those with a single infection. Also, the increasing infectious risk posed by HIV/AIDS might completely change the individual prognosis and the epidemiological features of leishmanial infection in this region of the world. Further prospective studies are needed to examine TB/VL co-infection, to examine the role of HIV in this matter and to investigate the clinical, diagnostic and therapeutic implications of the problem.

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