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Seroprevalence of Leptospirosis in Suspected High-Risk Sudanese Patients; A Pilot Exploratory Study

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ABSTRACT: The extent of leptospirosis is unknown in Sudan, and it might be mistaken for other more common febrile infectious diseases. Leptospirosis might also be associated with renal diseases that are common in Sudan. We intended to explore the existence of human leptospirosis in suspected high-risk patients in Khartoum, Sudan, via sero-screening random febrile patients and those undergoing renal dialysis. This pilot exploratory study was conducted over six months, from April to September 2013. Four hospitals were selected conveniently following a non-random sampling approach. One hundred nineteen febrile patients (with or without definitive diagnosis) and patients under renal dialysis were included, and their serum specimens and clinical and demographic data were collected. Sera were screened qualitatively for the existence of anti-leptospiral IgM antibodies using a rapid lateral flow immune-sorbent assay. Ethical clearance and official permissions were obtained. Of the 119 patients, 57 (47.9%) had end-stage renal disease. They were under dialysis at Renal Dialysis Unit in Asbab Charity Hospital in Bahri, 47 (39.5%) were febrile with unknown origin attending the Tropical Medicine Hospital in Omdurman, and 15 (12.6%) were febrile and were diagnosed as having malaria or typhoid and attended Yastabshiron Medical Centre and Bashauer Teaching Hospital. The prevalence of anti-leptospiral IgM antibodies among all 119 screened patients was 7%. The prevalence among the 57 with the end-stage renal disease was 9%, and among the 47 with a fever of unknown origin was 6%. The prevalence among the 15 with a fever of known origin (diagnosed as malaria or typhoid) was 0%. Almost all positive patients had recurring episodes of fever, were in close contact with livestock, were farmers, and had an untreated natural source for drinking water. Human leptospirosis does exist in this targeted population. It is probably a common febrile condition and can be easily considered one of the major causes of chronic kidney disease affecting people in Sudan. A national sero-screening for leptospirosis among those living in high-risk geographical areas and those at occupational risk is highly recommended.

Keywords: Febrile-Patients; leptospiral Infection; renal-dialysis; sero-screening; Sudan

INTRODUCTION

Leptospirosis is caused by pathogenic species of the genus Leptospira that belongs to the phylum Spirochaetes[1]. Leptospirosis is the most widespread, underreported, and neglected potentially fatal zoonotic bacterial infection affecting people worldwide[1,2]. It is estimated that more than 1.03 million cases and 58,900

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deaths occur each year worldwide[3]. The highest burden of leptospirosis affects the tropics and sub-tropics, especially following heavy rainfall and flooding[4].

Leptospirosis can be an acute infection and cause early complications like pulmonary hemorrhage and renal failure. It can also cause sub-acute and chronic complications and long-term sequelae[5]. Infection results from direct or indirect exposure to urine from infected reservoir host animals that shed pathogenic leptospires, which contaminate soils, surface waters, streams, and rivers[2]. Humans are infected through mucous membranes, abrasions, or cuts in the skin[6].

The laboratory diagnosis of leptospirosis can be established either by isolation of the intact live bacterium in specific culture media (which requires long incubation periods) or by detecting specific antibodies using serological techniques (the conventional ones are laborious and require freshly-made species-specific antigens)[7,8], or by detecting Leptospira DNA using molecular techniques[9]. Since IgM antibodies are detectable in the blood from 5 to 7 days after the onset of symptoms[2] and are relatively more manageable and accessible than culture and PCR, serology was mainly used to diagnose leptospirosis[2].

The diagnostic capacity of two commercially available tests, an ELISA IgM and a rapid immune chromatographic test IgG, were evaluated using archived samples collected from febrile patients in Thailand. IgM ELISA and ICT IgG sensitivities were 61% and 83%, respectively[10]. IgM ELISA was also used for screening in a large-scale study involving ten hospitals in North-Eastern Malaysia[11], where leptospirosis seroprevalence was identified in 8% (n=84) of sera collected from 999 febrile patients. Another ten-year retrospective seroepidemiological survey of leptospirosis in a tertiary care hospital in New Delhi[12] detected 391 (27%) positive cases by IgM ELISA. A qualitative sandwich immunoassay ‘’Leptocheck® WB Rapid Lateral Flow Immunosorbent Assay" was designed for the serodiagnosis of current or recent leptospirosis through detecting leptospira-specific IgM in human serum, plasma, or whole blood. This assay was evaluated by Sehgal et al. (2003)[13] in a primary health center in the Andaman Islands, where leptospirosis is endemic. All indices of validity and utility of lateral flow were similar to those of IgM ELISA and Lepto dipstick.

For many developing countries in general, there are no data on the prevalence of human leptospirosis, and for certain developing countries, the data may be considered unreliable[4]. In Africa, little is known; the few reports indicated the presence of the disease in Morocco, Eritrea, and Ethiopia, but with no specific data. Kenya[14] reported an outbreak, and Gabon[14] reported a 15% seroprevalence. The first report of the disease in Sudan was during the 70s in various animals, including camels, goats, sheep, dogs, and mice[15]. Except for the preliminary data on the association of leptospirosis with end-stage renal failure in Sudan[16], human leptospirosis has not been systematically studied in this country. We, therefore, intended in this study to explore the existence of human leptospirosis in suspected high-risk patients in Khartoum, Sudan. Because of its high validity and ease of use, and is highly recommended for limited-resource situations. Since this is a self-financed pilot study, we selected the qualitative sandwich immunoassay Leptocheck® WB rapid lateral flow immune-sorbent assay to sero-screen random febrile patients and those undergoing renal dialysis.

MATERIALS AND METHODS

This is a pilot exploratory study. It was conducted in several hospitals in Khartoum, Sudan, for six months, from April to September 2013. Hospitals were selected conveniently following a non-random sampling approach. A total of 119

patients were included; 57 of these suffered from end-stage renal disease and were under dialysis at the Renal Dialysis Unit in Asbab Charity Hospital in the Bahri area, 47 patients suffered from a fever of unknown origin and were admitted to the Tropical Medicine Hospital in Omdurman area. The remaining 15 patients were febrile, suffering from malaria or typhoid, and attending Yastabshiron Medical Centre and Bashauer Teaching Hospital in the Khartoum area.

Single blood samples were collected from the renal dialysis attendees, and sera were separated. Ready serum samples were collected from the laboratories of the remaining hospitals. Data were collected directly from patients undergoing renal dialysis; they were inquired about their age, sex, occupation, previous and current residence, contact with animals, source of drinking water, and the occurrence of previous episodes of fever. Data were collected from hospital records for patients attending the remaining hospitals.

Screening for specific anti-leptospiral IgM antibodies in the patient's sera was performed using the rapid lateral flow immune-sorbent assay Leptocheck® WB kit provided by Zephyr Biomedicals©. Sample storage and processing were performed at the serology laboratory of the Faculty of Medical Laboratory Sciences at the University of Medical Sciences and Technology (UMST) in Khartoum, Sudan. Informed consent was obtained from each patient at the renal dialysis unit before participating in the study. Permissions to use data anonymously and excess serum samples were obtained from hospital authorities. The ethical clearance for conducting this study was obtained from the Ethical Committee Board of UMST in Khartoum, Sudan, in April 2011.

Leptocheck® WB rapid lateral flow immune-sorbent assay

This is a qualitative sandwich immunoassay designed for the serodiagnosis of current or recent leptospirosis through detecting leptospira-specific IgM in human serum, plasma, or whole blood. The detection of leptospira infections caused by a wide range of different serovars is maximized using broadly reactive genus-specific antigens. The device's membrane test is pre-dispensed with anti-human IgM colloidal gold conjugate and leptospira genus-specific antigens at the test window (T). An anti-rabbit antiserum is pre-dispensed at the control window (C).

The kit components were brought to room temperature before use. After labeling, 10 µL of patient serum was transferred into sample port (A), five drops of running buffer were transferred to reagent port (B), and then the device was kept on the bench at room temperature for 15 minutes. Test results were then read as follows; A negative result is indicated when only one colored band appears in the control window (C). A positive result is indicated when, in addition to the band in the control window (C), another red to purple band appears in the test window (T). The later band indicates the presence of specific IgM antibodies to leptospira. The intensity of the test line depends upon the disease stage and the antibodies' titer in the patient serum. Known positive and negative controls were applied prior to testing to ascertain the device quality. In order to maintain valid test conditions and proper result interpretations, all recommendations mentioned in the kit leaflet were followed.

The ethical clearance for conducting this study was obtained from the Ethical Committee Board of the University of Medical Sciences and Technology in April 2011. Hospital authorities granted permission to collect specimens and data. Patients who were contacted directly consented prior to participation. Data obtained from hospital files were kept anonymous at all stages of the study.

RESULTS AND DISCUSSION

Of the 119 patients, 57 (47.9%) suffered from end-stage renal disease and were under dialysis at the Renal Dialysis Unit in Asbab Charity Hospital in Bahri in Khartoum, Sudan. Out of the 57, anti-leptospiral IgM antibodies were detected in 5 (8.8%). The average age of this group was 51+16 years (range 17 to 80 years), and males represented 60% (34/57). Their median age was 49 (range 42 to 67 years), and most (4/5, 80%) were males. All males (100%) were farmers. Except for one male, all 4 (80%) patients had a history of recurring episodes of fever and were living with domestic animals or had contacted them at some point. The sources of drinking water were as follows; the Nile for 3 out of the 5 (60%), chlorinated water for one (20%), and river canals for one (20%). Three (60%) of the five patients were originally residents in Northern and Central states (Dongola/Rufaa). Currently, in the Bahri area in Khartoum, one (20%) was resident in Juba and currently in the Omdurman area in Khartoum, and one (20%) was resident in Saudia Arabia in Khartoum. One (20%) of the positive patients had malaria at the time of sample collection; he was the one who was originally resident in Juba and uses river canals as a source of drinking water.

The second group of patients, 47 out of a total of 119 (39.5%), were febrile with an unknown origin; they attended the Tropical Medicine Hospital in the Omdurman area in Khartoum. Their average age was 40+16 years (range 14 to 65 years) and the male (46%) to female (54%) ratio was almost equivalent. Out of these 47 patients, anti-leptospiral IgM antibodies were detected in 3 (6.4%). All three positive patients were above 50 years old, and most (2/3, 67%) were males. All 3 had a history of recurring episodes of fever and were in close contact with domestic animals. The males were farmers and were living in Algazira state in Central Sudan. The female was a housewife living in Dar Alsalam in Khartoum. The third group of patients, 15 out of 119 (12.6%), were febrile and diagnosed with malaria or typhoid. They attended Yastabshiron Medical Centre and Bashauer Teaching Hospital. Sera from these patients were not reactive (0%) against anti-leptospiral IgM antibodies.

The prevalence of anti-leptospiral IgM antibodies among all 119 screened patients was 7%. The prevalence among the 57 with the end-stage renal disease was 9%, and among the 47 with a fever of unknown origin was 6%. The prevalence among the 15 with a fever of known origin (malaria/typhoid) was 0%. All findings are summarized in tables (1) and (2).

Table 1. Summary of the Overall Findings

Category	End Stage Renal Disease (Under Renal Dialysis)	Fever with Unknown Origin	Fever with Diagnosis (Malaria/Typhoid)	Total
Average Age	51±16 Years (Range 17 to 80)	40±16 Years (Range 14 to 65)	-- *	
Sex	34 Males 23 Females	12 Males 14 Females **	-- *	
Total Number of Patients	57 (47.9%)	47 (39.5%)	15 (12.6%)	119 (100%)
Prevalence of Anti-Lepto IgM Antibodies	5 (8.8%)	3 (6.4%)	0 (0%)	8 (7%)

*Data is not available

**Data is only available for 26 cases (21 are missing)

Table 2. Data on Patients with Positive Anti-Leptospiral IgM Antibodies

No	Sex	Age (years)	Occupation	Contact with Animals	Previous Episodes of Fever	Source of Drinking Water	Study Groups
1	Male	65	Farmer	No	No	Well/Nile	End Stage Renal Disease
2	Male	42	Farmer	Yes	Yes	Canal	
3	Male	67	Farmer	Yes	Yes	Nile	
4	Male	49	Farmer	Yes	Yes	Chlorinated	
5	Female	42	Housewife	Yes	Yes	Nile	
6	Female	65	Housewife	No	Yes	Well/Nile	Fever with Unknown Origin
7	Male	52	Farmer	Yes	Yes	Canal	

* Data on case number 8 was missing

Chronic kidney disease, which is a state of kidney damage or reduced kidney function lasting for three months or longer, is common in Sudan[17]. Several conditions can cause chronic kidney disease, including diabetes, hypertension, or glomerulonephritis[18]. Leptospirosis is associated with kidney disease[2], but it has never been thoroughly investigated and is severely overlooked in most African countries, including Sudan. We believe this bacterium could be a significant cause of chronic kidney disease in the country. In addition, symptoms of leptospirosis may mimic those of other endemic infections in Sudan, such as malaria, typhoid, meningitis, hepatitis, or dengue[2]. We, therefore, intended to explore its existence in possibly suspected high-risk patients in Khartoum via sero-screening random febrile patients and those undergoing renal dialysis.

Interestingly, anti-leptospiral IgM antibodies were detected in 7% out of all our 119 screened patients (Table 1). Similarly, clinical researchers in Vanuatu (Pacific Islands)[19] investigated leptospirosis as a cause of non-malarial acute febrile illness and found a high seroprevalence of 6%. Leptospira seroprevalence in a study in Western Uganda was 35%[20]. They reported a probable recent leptospirosis seroprevalence of 1.9% associated with self-reported malaria within the past year[20]. Other studies reported varied leptospiral seroprevalence of 8.4% among febrile inpatients in North-Eastern Malaysia[21], 24% among individuals in high-risk occupations in Morocco[22], and 38.5% among miners in India[23]. Further, a study in Portugal revealed that 46% of the Sa˜o Miguel Island population has to circulate anti-leptospiral antibodies[24].

9% out of all our 57 patients in renal dialysis and 6% out of the 47 patients with fever of unknown origin were seropositive for leptospiral specific IgM (Table 1), indicating current or recent leptospirosis. Unfortunately, all patients undergoing dialysis were at end-stage renal disease, and those with a fever of unknown origin were long-term hospitalized. Knowing that the condition could have been easily reversed if the highly antimicrobial susceptible bacterial cause was identified early and treated is heartbreaking. Therefore, sero-screening suspected febrile patients or those with kidney disease should be a must in hospitals and renal centers in Sudan. Since this lateral flow immunochromatographic test kit can provide reliable results and is easy to perform, rapid can be readily available, and cost-effective, we recommend introducing it in the routine diagnostic laboratories, outpatient clinics, and rural health centers in Sudan.

Most human cases of leptospirosis worldwide result from occupational exposure to water (or flood waters) or soil contaminated with animal urine[2,25]. People with a relatively high incidence of infection–amongst others-are farmers, agricultural

workers, animal handlers, and those exposed to flood waters[2,25]. We, therefore, intended to explore these risk factors. 80% of the seropositive patients with end-stage renal disease in our study had a history of recurring episodes of fever and were living with domestic animals or had contacted them at some point (Table 2). Most of these patients were farmers, and contaminated food and drink with animals and rodents' urine can never be ruled out. These factors indicate and support the diagnosis of leptospirosis. In addition, the source of drinking water was the Nile River for 60% of these patients, and 20% used river canals (Table 2). Of the original residents, 60% were Dongola and Rofaa, and 20% lived in Juba. Considering that people living in these areas rely on the mentioned natural water sources without prior treatment suggests another possible risk for acquiring the infection. Higher risk activities in the study from Western Uganda included skinning cattle and living close to monkeys[20]. Whereas most of the seropositive high-risk individuals in the study from Morocco[22] were involved in poultry (37%), market fish (26%), and meat slaughterhouse workers (15%).

Furthermore, the possibility that our seropositive patients with fever of unknown origin might have consumed contaminated water by animal and rodents' urine significantly arises since they were living in Aljazira and Dar Alsalam. The first area is known to be agricultural, facilitating a rich environment for the survival and spread of the disease, rural residents have urbanized the second area, and the different water facilities have less quality control. All positive patients were middle-aged or elderly (Table 2). Many studies also reported a higher prevalence in this age group[2,15,25]. The male-to-female ratio was almost equivalent, consistent with Rafizah et al. (2012)[21] findings in their large-scale study in North-Eastern Malaysia.

In the present study, we identified co-infections in 25%; one was co-infected with malaria and one with hepatitis C. However, we did not identify anti-leptospiral antibodies (0%) in the 15 patients with fever of known origin (i.e., malaria/typhoid). On the other hand, combined infections have been reported in the literature[2,25]. Chaudhry et al. (2013)[12] reported hepatitis-E, malaria, and dengue fever in 7% of patients with leptospirosis.

Leptospirosis renal complications are present in our population, indicated by seropositivity of approximately 2:1 among those with end-stage renal disease (i.e., 62%) relative to those with a fever of unknown origin (i.e., 38%). Yang et al. (1997)[26] reported a high prevalence of renal failure among patients with leptospirosis; they also reported fever and jaundice. Because of their finding, Yang and colleagues recommended that leptospirosis be suspected in febrile patients with jaundice and renal failure. A major limitation in this study was the scarce resources and lack of funding.

CONCLUSION

Human leptospirosis does exist in this targeted population. It is probably a common febrile condition and can be easily considered one of the major causes of chronic kidney disease affecting people in Sudan. A national sero-screening for leptospirosis among those living in high-risk geographical areas and those at occupational risk is highly recommended.

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CONFLICT OF INTEREST

The author has declared no conflict of interest.

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