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The Incidence of *Leishmania donovani* using RK39 and Buffy Coat Concentration Technique in Blood Donors in Gadarif State

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ABSTRACT

Visceral leishmaniasis is an endemic disease in Sudan which affects many populations certainly in Gadarif State. A hospital-based cross-sectional study was conducted at Gadarif Teaching Hospital, 100 healthy apparent blood donors were included in the study and their blood specimens were examined microscopically by buffy coat technique and serologically by rk39 and there was no positivity reported in the study. Further study must be conducted with large sample size and molecular diagnostic method (PCR) must be used.

Keywords: Kala-azar, *Leishmania infantum*, Visceral leishmaniasis, Blood donors

INTRODUCTION

Kala-azar is considered as one of the most important neglected vector-borne zoonotic parasitic sicknesses with a high mortality rate if left untreated. Sandfly is the vector, man and carnivores are the typical reservoirs. In the human host, parasites are surrounded by macrophages, which are then carried by the circulatory system to other organs like liver, spleen and bone marrow where they cause hyperplasia and or macrophage cells [1].

World Health Organization (WHO) estimates that the disease is endemic at least in 60 countries of 4 continents with 0.5 million new cases and 40,000 cases of death annually [2]. The immense majority (more than 90%) of cases are in Sudan, India, Bangladesh, Nepal, and Brazil.

Visceral leishmaniasis remains hidden in persons with normal immune systems and appears to be very common in most of the VL endemic foci [3]. Visceral leishmaniasis is caused by *Leishmania donovani* complex including *Leishmania infantum* and *Leishmania donovani*. In general *Leishmania infantum* infections are asymptomatic, raising concern that the parasite could be available in blood donors from else healthy inhabitants in regions to which it's endemic [3]. The transmission of parasites through blood transfusion is relatively rare. Currently, 6 parasitic infections are yet considered as Transfusion-transmitted infections (TTI) including *Plasmodium spp, Trypanosoma cruzi, Leishmania spp, Babesia microti, Toxoplasma gondii* and Filaria [4].

The occurrence of asymptomatic carriers among blood donors has been carried out in southern Europe, with a proportion ranging from 0-36.4% depending on the test used and the number of individuals studied [5-17]. Several studies suggest that the presence of *Leishmania* in the peripheral blood of asymptomatic carriers is probably episodic, suggesting that such individuals are asymptomatic carriers for a variable period [18-43].

The confirmation of human cases of Transfusion transmitted leishmaniasis (TTL) is of interest, certainly VL, with clinical features and outcomes similar to those of the natural infection, not only in endemic but also in non-endemic areas [44]. Transmission of *Leishmania* by transfusion is most likely to happen in an endemic region because that is where infected donors most often reside. In those areas, a case of transfusion transmission is frequently impossible to differentiate from local transmission by sandflies. That and the fact that visceral infection with *Leishmania* is asymptomatic in healthy individuals is expected to result in underestimation of the occurrence of transfusion-transmitted infection. *Leishmania* does produce clinical disease in infants and the immunocompromised as demonstrated in documented cases of transmission by transfusion in Chung and colleagues [45]. An additional reason for the low number of reported cases is that clinical suspicion is low in non-endemic areas where the symptomatic infection is

often labeled as fever of unknown origin because diagnosis is difficult without a high index of clinical suspicion and a specific search for *Leishmania*. An additional level of complication for the donor pool is presented by the fact that transplacental and sexual transmission is possible [46,47].

The USA organization of Blood Banks has recommended a ban on blood donations from a chosen group (soldiers coming to the United States from VL endemic areas, certain countries of Middle East) to prevent Transfusion transmitted Leishmaniasis [48].

Rationale

Visceral leishmaniasis is a major health problem in Sudan with the highest disease burden in Gadarif State. In addition to the basic route of transmission via the sandfly bites, leishmaniasis may also be transmitted through blood transfusion. No information is available about the prevalence of *Leishmania* infection between blood donors in Sudan; therefore we aimed to investigate this question.

Objectives

General objectives: To evaluate the prevalence of asymptomatic *Leishmania* infection among blood donors in Gadarif State, Sudan.

Specific objectives:

- To compare between the sensitivity of buffy coat and techniques rk39
- To compare the specificity of rk39 and buffy coat technique

MATERIALS AND METHODS

Study Area

Gadarif State is one of the most significant states in Sudan and plays a significant role in the economic and agricultural activities of the country. It has inhabitants of more than 1727404 residents, cover 75000 km^2, and lies between latitude 14 and 16 north and longitude 33 and 36 east. The average rainfall is 612/millimeter. Four rivers pass through the State (Atbara, Elrahad, Saitit, and Basalam) and there are many forests, which provide an excellent environment for the sand fly vector. There are 10 clinics for the diagnosis and treatment of VL in the State.

Study Design

Hospital based-descriptive cross-sectional study.

Sample Size

Total of 100 healthy-appearing males blood donors.

Study Period

The study period was from January 2018 to April 2018.

Study Population

Blood donors attended to the blood bank at Gadarif teaching hospital during the period of the study (January, 2018-April, 2018).

Inclusion Criteria

Only persons with normal blood pressure, normal hemoglobin level and free from HIV, hepatitis B, and C viruses were included in the study.

Exclusion Criteria

Each person with abnormal blood pressure, abnormal hemoglobin level, HIV positive, hepatitis B or C positive and those who matched the WHO clinical features of visceral leishmaniasis (prolonged irregular fever, splenomegaly, and weight loss), will be excluded from the study.

Ethics

The study received ethical clearance from the research ethical committee, Alneelain University.

Method

Collection of blood: Under all aseptic safety measures (cleaning the area with povidone-iodine followed by adequate rubbing by alcohol pad containing 60% isopropyl) 5 ml of venous blood was collected from median ante-cubital or appropriate veins by gentle suction. The collected blood specimens were equally divided immediately into plain vacutainer and a 3.2% Tri-sodium citrate coated vacutainer. Immediately after collection with proper labeling, blood was carried to the laboratory for buffy coat preparation and serum.

Serum preparation: About 2.5 ml of whole blood was added in an untreated tube for serum. Blood was allowed to clot for 20-30 min at room temperature followed by centrifugation at 2,500 rpm for 5 min. The serum was removed from the clot and collected in cryogenic vials and labeled consequently.

Buffy coat (density gradient centrifugation): In a germ-free round bottom disposable test tube (10 ml capacity), 2 ml of citrated blood from the vacutainer was poured slowly by the side of the test tube so that the blood completely cover the separation fluid without any mixing. The test tube was centrifuged for 15 minutes, at 3000 rpm referable in a swinging centrifuge engine. Following centrifugation, a thick buffy coat was seen in the interface of plasma. RBCs were settled at the bottom part of the test tube. At the moment the plasma was gently sucked by a micropipette of 1 ml capacity and was preserved in an Eppendorf tube. Then by another of 100-200l capacity/micropipette, the buffy coat material was sucked out very gently [49].

Staining of buffy coat smears: Buffy coat films were stained by Leishman stain.

Process of Leishman staining: An air-dried thin film was made and placed on a staining rack and flooded with Leishman stain. The slide was left for 2 minutes to fix. Two times amount distilled water was added over the flooded stain from a plastic wash bottle for better mixing of the solution. The stain was left for 10 minutes. The stain was washed with running tap water and air-dried again the smear to examine under oil immersion objectives [49].

Microscopic examination of the buffy coat film: As a minimum of 1000 fields per slide were examined under oil immersion lens to detect amastigote form of *Leishmania donovani*. Amastigotes appear as round or oval bodies measuring 2-3 m in length and are found in inside or outside the monocytes and macrophages. In preparations stained by Leishman stain, the cytoplasm appears pale blue, with a relatively large nucleus that stains red. In the same plane as the nucleus, but at a right angle to it, is a deep red or violet rod-like body named a kinetoplast. If any amastigote in 1000 fields was detected, the slide was reported as positive.

rK39 Rapid Test

We applied a ready-to-use Immunochromatographic strip manufactured by InBios Inc. (Seattle, WA). This strip has rK39 antigen immobilized as the lower band of the nitrocellulose pad of the strips, which contain protein A/colloidal gold as a detection reagent [50]. A band of 1 cm above the rK-39 band contained antibody to protein A/colloidal gold and was used as a positive control to detect normal immunoglobulin G (IgG).

The rK39 immunochromatographic RDT, Kala-azar Detect (InBios International, USA) was achieved at RMRIMS in accordance with manufacturer's instructions. At room temperature, 20 µl of serum was prepared from venous blood or one drop of finger stick blood was added to the dipstick. A single drop of blood was applied in this study because this is what is normally performed in the field. Three drops of the chase buffer solution were added to a test tube followed by addition of the dipstick into the test tube including the chase buffer. The outcomes were read after 10 minutes. The test was regarded as positive when both the control line and the test line showed red in color.

RESULTS

There are no positive cases among the study population (100% males) detected either by Buffy coat technique or rk39 rapid test (Table 1).

Table 1 Frequency table

Label	Value	Freq	%	Sum %
r1(sample size)	100	0	0%	0%
1		0	100%	
categories		cases		
Mean: 0; Median: above purple row; Sum: 0; Cases-N: 0; Variance: 0; sd: 0; se: 0; 95% CI: 0>0>0				

DISCUSSION

Our result is compatible to the result of many studies conducted in southern Europe of many investigators done and incompatible with the result of the study done by Sarkari, et al., showed that; 28 blood donors (1.4%) were positive for *Leishmania* infection by DAT. Only one of these seropositive donors was positive for *Leishmania* infection by polymerase chain reaction also our result differ from that obtained by Fukutani, et al., which showed that, anti-Leishmania serology by ELISA was positive in 5.4% (74.5% of the study population was males) due to differences of species (*Leishmania donovani*/*Leishmania infantum*), techniques, sample size, gender of the study population and personnel experiences.

CONCLUSION

We conclude further studies must be conducted with more sample size, including both sexes (males and females) and additional diagnostic techniques must use chiefly Polymerase chain reaction (PCR).

DECLARATIONS

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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