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Seroprevalence of Kala-azar in El Hawata district, Eastern Sudan

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Abstract
A study on Seroprevalence of Kala-azar was conducted in El Hawata District, Eastern Sudan. A group of 399 individuals were participating in the study. They were interviewed using a questionnaire, and then a sample of blood was taken from each. These samples were centrifuged to separate sera for performing Direct Agglutination Test (DAT) to detect anti leishmania bodies in the serum specimen. The study revealed that, Seroprevalence of Kala-azar at a cut off ≥ reciprocal titre of 3200 was found to be 17.2%. In the study findings, c was high in males (9.4%) compared to 7.7% in females (Odds Ratio (OR) = 1.2402, 95% Confidence Interval (CI) from 0.5983 to 2.5709). It was found that the highest seroprevalence (11.7%) was seen in age group 31 – 40 years followed by 21 – 30 years (7.3%) with a statistical significance (X^2 =7.778, P value = 0.0508). As a conclusion, Kala-azar seroprevalence was high and the area still suffering from the disease.

Keywords: seroprevalence, Kala-azar, agglutination, leishmania, Sudan.

Introduction
Kala-azar, is a vector-borne disease, caused by leishmania parasite namely L. donovani, L. Chagasi or L. infantum. It is endemic disease in large areas of the tropics, subtropics and the Mediterranean basin (François *et al*, 2007) [1]. It is transmitted by infected female sand fly. The disease is also named as, "black fever" in Hindi, due to darkening of the skin and prolonging fever. The main signs are fever, enlargement of the spleen and liver, anaemia. Rarely, Jaundice, oedema, psychiatric illness, and neurological changes may occur (Mohammed *et al*, 2014) [2].
Visceral leishmaniasis (Kala-azar) is endemic in several countries and Sudan is considered to be one of the main foci of Kala-azar in the world (Omran *et al*, 2000) [3].
From the early 1900s, visceral leishmaniasis (VL; kala-azar) has been among the most important health problems in Sudan, particularly in the main endemic area in the eastern and central regions. Several major epidemics have occurred, the most recent--in Western Upper Nile province in southern Sudan, detected in 1988--claiming over 100,000 lives (Zijlstra and el-Hassan, 2001) [4]. In the past, several outbreaks of the disease occurred in Sudan. In the 1950s, an outbreak of VL was reported from El-Jazeera Abba north to Kosti in the central part of Sudan on the eastern bank of White Nile (Sally *et al*, 2012) [5].
Delays and difficulty in diagnosis are common due to the long incubation period of the agent, nonspecific symptoms, and the difficulty of identifying the intracellular protozoa (Donovan bodies) in tissue aspirates. It is usually diagnosed by serology or bone marrow examination (Mustafa, 2014) [6].
Sensitivity of Direct Agglutination Test (DAT) as a diagnostic tool for Kala-azar was high in Sudan (94%) and India (92.3%) but low in France being 88.5%and 54.5% for visceral leishmaniasis (VL) and VL/HIV patients, respectively (Elfadil Abass *et al*, 2015).
Visceral leishmaniasis or Kala-azar is fetal disease if it is not treated. Most antileishmanial drugs are highly toxic, and need hospitalization. In the recent years, a combined therapy resulted in reduction of time and cost has been achieved (Lucio *et al*, 2012) [8].

Materials and Methods
Study design
The study was a cross sectional descriptive study which carried out in El Hawata Town, Eastern Sudan.

Study area

El Hawata is important town in Gedaref state, eastern Sudan. It is an agricultural area where people grow Dura, sesame, dockhon, and groundnuts, in addition to some indigenous trees are *Acacia Senegal* (hashab), *Balanitis aegyptica* (higleeg), *Acacia mellifera* (kiter), *Acacia seyal* (taleh), and *azadirachta indica* (neem). The climate of the area is poor savannah. The year is sharply divided between the rainy season, June-October, and the dry season, November-May. The temperature usually is ranging between 21.9 °C and 42.9 °C in the dry season while in the rainy season between 22.5 and 38 °C. These conditions provide suitable environment for the vector Phlebotomus sand fly to survive and multiply.

Study population

The area was populated by about 118398 persons. The study was conducted among residents of the town who are descend from many ethnic groups, such as Bargo, Benamer, Fallata, Masaleet, etc.

About 399 individuals were calculated by certain sampling equation and chosen by multi stage stratified sampling technique.

Ethical clearance

Ethical approval for this study was obtained from the Ministry of Health, Gedaref state, Eastern Sudan. An informal consent of participants was obtained before data collection process.

Data collection

1- Interview: A questionnaire was used to collect socio-demographic data from each individual in addition to samples for serological and microscopic examinations. These data implied basic, socioeconomic, environmental and medical information

2- Direct Agglutination Test (DAT): To perform Direct Agglutination test about 3ml of blood were taken by a normal syringe from all participants. The sera were separated and transferred. To prepare 100 ml of diluents, about 0.2mg of gelatin was added to 100 ml of normal saline and heated in water bath at 56oc for 5 minutes to dissolve gelatin completely. By a micropipette, 0.8µl of ß-mercaptoethanol was added to mixture (gelatin plus normal saline). Two µl of the blood serum were diluted by100µl of the prepared diluent in a tube. About 50µl of the prepared diluent were poured in each well of V-shaped microtitre plate multichannel pipette except well 2, in with other 50µl were added. Two-fold dilution series of sera were made in the V-shaped microtitre plate, starting at dilution of 1:100 (well 2) and going up to a maximum serum dilution of 1:102.400 (well 12). Well 1 was used as a negative control. Fifty µl of liquid antigen (concentration of 5×107 parasite per ml) were added to each well containing 50µl diluted serum. Finally the plate was carefully shaken, covered and allowed at room temperature. The results were read after about 18 hours of incubation. Agglutination was visible as blue/purple mat in the wells of the microtitre plate. The cut-off value of the DAT was set at 1:3200.

The data were analyzed by SPSS and Seroprevalence, Chi square, P value, Odds Ratio (OR), and 95% confidence interval of odds ratio, were calculated.

Results

Table 1 shows that the Seroprevalence of visceral leishmaniasis in El Hawata Town, Eastern Sudan was 64 (17.2%). In the study findings, seroprevalence was high in males (9.4%) compared to 7.7% in females (Odds Ratio (OR) = 1.2402, 95% Confidence Interval (CI) from 0.5983 to 2.5709) as shown in table 2. In table 3, the highest seroprevalence (11.7%) was in age group 31 – 40 years followed by 21 – 30 years (7.3%) with a statistical significance (X^2 =7.778 P value = 0.0508).

Table 1: Seroprevalence of visceral leishmaniasis in El Hawata Town, Eastern Sudan. (n = 399)

DAT result	No	%
Positive	35	8.8
Negative	364	91.2
Total	399	100

Table 2: Sex distribution of visceral leishmaniasis in El Hawata Town, Eastern Sudan. (n = 399)

Sex	DAT result		Total
	Positive	Negative	
	No (%)	No (%)	No (%)
Male	23 (9.4)	221 (90.6)	244 (61.2)
Female	12 (7.7)	143 (92.3)	155 (38.8)
Total	35 (8.8)	364(91.2)	399 (100)

Odds ratio = 1.2402 95% Confidence Interval (CI) from 0.5983 to 2.5709

Table 3: Age distribution of visceral leishmaniasis in El Hawata Town, Eastern Sudan. (n = 399)

Age (years)	DAT result		Total
	Positive	Negative	
	No (%)	No (%)	No (%)
10-20	2 (33.3)	4 (66.7)	6 (1.5)
21-30	13 (7.3)	164 (92.7)	177 (44.4)
31-40	16 (11.7)	121 (88.3)	137 (34.3)
41-50	4 (5.1)	75 (94.9)	79 (19.8)
Total	35 (8.8)	364(91.2)	399 (100)

X^2 =7.778 P value = 0.0508

Discussion

Kala-azar or visceral leishmaniasis is one of endemic disease in eastern Sudan. This present study as well as other studies confirmed the endemicity of the disease in these areas. It is a public health problem in Gedaref state since many years ago. It is necessary to gather enough information about magnitude, pattern and mapping of disease for effective control measures on a large scale. This present study is an attempt to measure the problem in one town, and definitely several studies are needed in different areas in eastern Sudan from time to time to give more recent picture about the kala-azar situation. In the present study the seroprevalence was 8.8%. The study used Direct Agglutination Test (DAT) due to its high sensitivity to sort out kala-azar cases. In several studies the sensitivity of DAT was ranging between 94% and 100% (Omran *et al*, 2000) [3]. It was relatively high and expected to affect a large segment of population. Seroprevalence rates of Kal-azar in some previous studies were measured in certain areas in Gadaref state e.g in Rahad region was obtained 33.9%, in Atbara River region 21.6% and in Gadaref town 10.6% (Elnaiem *et al*, 2003) [9] but these studies were conducted more than ten years ago and now the situation is changed due to programme control efforts. Also it was lower when it is compared with data mentioned in a

study about leishmaniasis in three villages in Rashad Province, West of Sudan which was found to be 51.2% of study population was positive when they subjected to Leihmanin Skin Test (Amani, 2011) [10].

Walter *et al* (2013) [11] found that seroprevalence of kala-azar using DAT in Pokot county, Amudat district, Northeastern Uganda was 17.2%. In Iran an Anti-Leishmania antibody was detected in 50 out of 1628 children (3.1%) by direct agglutination test (antibody titre ≥ 1:3200) (Sarkari *et al*, 2010) [12]. Because Kala-azar could be asymptomatic in many cases, some researchers investigated asymptomatic cases of the disease using different diagnostic techniques and measured prevalence accordingly. One of these studies was carried out in Iraq and the prevalence was found to be 34.4% using rk39 (Haidar and Faris, 2013) [13]. High prevalence rates in endemic areas usually include both symptomatic and asymptomatic.

According to the findings in this study kala-azar was more frequent in males than females (Odds ratio = 1.2402) and people in age group 10-20 years were more affected with kala-azar. Males between 5 and 14 years of age were the most affected group (El-Safi *et al*, 2004). The highest rate of infection (5.2%) was in the age group 10 years (Sarkari *et al*, 2010) [12]. In a study implemented in villages of eastern Gedaref state, Sudan, the male female sex ratio was 1.08 and the median age was 15 years (Yolanda *et al*, 2012) [15].

Conclusion

Kala-azar is still a public health problem according to the findings in this community-based study, particularly among youth and young people. Urgent effective control measures are needed to minimize the problem.

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