Seroprevalence Of Visceral Leishmaniasis Using Direct Agglutination Test (DAT) In Tabark Allah Village, Eastern Sudan, 2010.

Mohammed A Elawad, Kamil M Ali, Abdelsafi A gabbad, Omran F Osman, Ahmed M Musa

Absract: Visceral Leishmaniasis (VL) is a protozoan disease caused by Leishmania donovani and transmitted by phlebotomus sand fly. The disease is endemic in Sudan and large area of tropics. It has been reported in eastern Sudan since the beginning of the twentieth century. This is a cross-sectional study conducted in Tabark Allah Village, eastern Sudan to measure the seroprevalence of visceral Leishmaniasis. Samples of blood sera were collected from 373 individuals selected by systematic random technique. The samples were examined using Direct Agglutination Test (DAT) to detect the anti-leishmania antibodies. Demographic and socio-economic data were obtained by direct interview with the study group using a pre-tested questionnaire.The seroprevalence at a cut off ≥ reciprocal titre of 3200 was found to be 17.2% which was more prevalent among children (70.2%) with p value of 0.000, compared to adults. Family income had a significant association with the seroprevalence of visceral Leishmaniasis (p value: 0.05).

Key words: direct agglutination, donovani, endemic, leishmaniasis, seroprevalence, visceral,

INTRODUCTION

Visceral leishmaniasis (VL) is a parasitic disease caused by Leishmania donovani complex and transmitted by infected female sand fly (3). The disease is also named as kala azar (KA), “black fever” in Hindi, due to darkening of the skin and prolonging fever (14). The disease is characterized by fever, enlargement of the spleen and liver, anaemia (4). Rarely, Jaundice, oedema, psychiatric illness, and neurological changes may occur (27). The case-fatality rate among untreated cases might be more than 90% (6). About 90% of visceral cases occur in Bangladesh, Brazil, India, Nepal and Sudan(7). In Sudan the disease has been reported in Sudan since the beginning of the twentieth century, particularly, in the eastern Sudan (27). In addition to the role of sand fly in the disease transmission, visceral leishmaniasis can be transmitted by transfusion of infected blood, needle sharing, sexual intercourse, (24). Congenital transmission of visceral leishmaniasis is rare (27). Dogs, jackals, monkeys and foxes are considered as reservoirs of VL (20,27,13). The disease is more frequent in children than in adults (15,8). Males are more affected than females (23,18,22,1,16,19). Poverty is associated with visceral Leishmaniasis as the disease spread more in poor people (5,18,26). The diagnosis of VL is based on demonstration of the parasite in smears obtained from bone marrow, lymph nodes, spleen and liver (21), while serodiagnosis done by a number of serological techniques (2). Direct Agglutination Test (DAT) is a suitable tool for the sero-diagnosis of visceral leishmaniasis under field or rural conditions (9). Several previous studies found that sensitivity of DAT is ranging between 91% and 100% (24). Despite the high sensitivity the DAT cannot distinguish between active disease, sub-clinical infection or past infection (27). Vector control and community awareness are effective measures of VL control (3).

MATERIALS AND METHODS

Study design and population

It was a cross-sectional household study included 373 of Tabark Allah villagers. They were selected by systematic random sampling.

Study area

Tabark Allah is located at approximately 120 km south Gedaref town, eastern Sudan. It is populated by 5355 of people who are belonging to different tribes such as Bargo, Bederia, Benamer, Benhelba, Benrashid, Dajo, Fallata, Gemir, Housa, Marareet, Masaleet, Meseiria, Rezigat, Tama, and others. The area is agricultural where the agriculture is the main Occupation.

Ethical clearance:

Ethical approval for this study was obtained from the Ministry of Health, Gedaref state, Eastern Sudan. The consent of participants was obtained verbally.

Data collection methods

Direct Agglutination Test

Approximately 3ml of blood were taken by a normal syringe from all participants. The sera were separated and transferred from study area to Khartoum in an ice container. To prepare 100 ml of diluent 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 narmal saline). Two µl of the blood serum were diluted by100µl of the prepared diluent in a tube. Using the multichannel pipette. 50µl of the prepared diluent were poured in each well of V-shaped microtitre plate(observation: additional 50µl were poured in well 2). 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.

Interview

Participant or head of the household was interviewed. Specific questionnaire was designed to collect demographic and socio-economic data.

Data analysis

Collected data were analyzed using SPSS software package and different statistical tests were used to determine a significance of various variables in kala-azar infection.

RESULTS

A total of 373 individuals were involved in this study. The socioeconomic characteristics of the sampled population are shown in Table 1. Only 9(2.4%) were under five years, 78(20.9%) were 4 – 14 years, 98(26.3%) were 15 – 24 years, 74(19.8%) were 25 – 34 years, 48(12.9%) were 35 – 44 years and 66(17.7%) were 44 - 65 years. The study group comprised 192(51.5%) males and 181(48.5%) females. With regard to educational level, approximately half of the study population had basic level of education. About 146(39.2%) had no formal education and 38(10%) were either secondary or university. The majority of respondents were unskilled labourer 151(40.4%) followed by students 123(33%). The majority of our sample 152(40.8%) had monthly income over 300 SDG and only 29(7.8%) had less than 100 SDG monthly income Table 2 shows that the seroprevalence was 64 (17.2%) using direct agglutination test (DAT). Table 3 shows distribution of DAT positive and negative people by age. The highest seroprevalence (35.9%) was in children in age group 5 – 14 years, and (33.3%) in under five children. The table shows statistical significance (p 0.000). In table 4, the highest percentage of DAT positive (27.9%) was found among families with 100 - 200SDG monthly income, >300SDG was 16.4%, 201 – 300 was 13.7%, less than 100SDG was 10.3%. According to analysis there was statistical significance between monthly income and positive DAT ($x^2 = 7.601$ and $p = 0.05$).

Table (1): socio-demographic profile of the study population, in Tabark Allah village, Gedaref state 2010. (n=373)

		Number	%
Age	< 5	9	2.4%
	5 – 14	78	20.9%
	15 – 24	98	26.3%
	25 – 34	74	19.8%
	35 – 44	48	12.9%
	45 – 65	66	17.7%
Sex	Male	192	51.5%
	Female	181	48.5%
Education	no schooling	146	39.2%
	basic	189	50.7%
	secondary, university	38	10%
Occupation	Student	123	33%
	unskilled labourer	151	40.4%
	Farmer	72	19.3%
	Employee	8	2.1%
	Merchant	19	5.1%
Monthly income	< 100 SDG	29	7.8%
	100 – 200	68	18.3%
	201 – 300	124	33.2%
	> 300 SDG	152	40.8%

Table (2): Seroprevalence of visceral leishmaniasis, using direct agglutination test (DAT), in Tabark Allah village, Gedaref state 2010. (n=373).

DAT	No	%
positive	64	17.2%
negative	309	82.8%
Total	373	100%

Table (3): Age distribution of DAT positive people, in Tabark Allah village, Gedaref state 2010. (n= 373)

Age	Direct agglutination test (DAT)					
	positive		negative		Total	
	No	%	No	%	No	%
< 5	3	33.3%	6	66.7%	9	2.4%
5 – 14	28	35.9%	50	64.1%	78	20.9%
15 – 24	23	23.5%	75	76.5%	98	26.3%
25 – 34	6	8.1%	68	91.9%	74	19.8%
35 – 44	1	2.1%	47	97.9%	48	12.9%
45 – 65	3	4.5%	63	95.5%	66	17.7%
Total	64	17.2%	309	82.8%	373	100%

X2 = 42.99 P = 000

Table (4): Relationship between monthly income of study group and the result of DAT, in Tabark Allah village, Gedaref state 2010. (n=373)

Monthly income SDG	The result of DAT					
	positive		negative		Total	
	No	%	No	%	No	%
<100	3	10.3%	26	89.7%	29	8.8%
100 – 200	19	27.9%	49	72.1%	68	18.2%
201 – 300	17	13.7%	107	86.3%	124	33.2%
>300	25	16.4%	127	83.6%	152	33.8%
Total	64	17.2%	309	82.8%	373	100%

X2=7.601 p=0.05

DISCUSSION

The findings indicate that the seroprevalence of visceral leishmaniasis was 17.2% using direct agglutination with cutoff point of 1 : 3200. We considered anti-Leishmania antibodies titers at ≥ 1:3200 as positive DAT in this investigation. However some studies used different cutoff point e.g 1 : 6400 (17). This seroprevalence is, relatively, high. It is lower than the seroprevalence achieved by El Safi et al (2004) which was 66% when they used leishmanin skin test to investigate visceral leishmaniasis in Marbata village, Gadaref state (12). According to previously published data, seroprevalence of visceral leishmaniasis was recorded high 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% (10). Although this seroprevalence was considered high, it is similar in many endemic areas, e.g. in Bihar, India, 18% were DAT positive (25). Also seroprevalence in the present study is lower than seroprevalence (using DAT) conducted in Barbar El Fugara village, Gadaref state from 1996 to 1997, which was 26.4% in schools and 8.9% in a survey conducted in households (11). There was statistical significant association between visceral leishmaniasis and age of individual (p = 000). Generally, The children were more affected than adults. Children under 15 years had highest percentage in this serological investigation. This result agrees with findings of several studies which they mentioned that the disease is more frequent in children e.g. In Um-Salala village, Sudan, Zijlstra.E.E. and el-Hassan (2001) found that 50% of infected individuals were in age group 5 – 15 (27). In Venezuela, 80.6% of infected persons were younger than 15 years (22). This study revealed that statistical significance was found between seroprevalence and family income (p = 0.05). Such finding also achieved in a study

conducted in Bangladesh (5).

CONCLUSION

Seroprevalence of visceral Leishmaniasis was high, particularly among children. Demographic and socio-economic status might affect the frequency of the disease.

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