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Short Communication

The Sero-Prevalence of HIV-1 among Visceral Leishmaniasis Patients in Damazen Town the Blue Nile State Sudan

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Abstract

The aim of this study was to determine HIV-1 seropositivity among visceral leishmaniasis (VL) patients. It was also used to compare between ELISA, WB and RT-PCR as diagnostic test tools. The study was conducted on 200 patients and 200 healthy controls from Blue Nile State. We used Indirect Enzymatic-Link Immunosorbent Assay (ELISA), Western Blot Test and Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) as diagnostic test. The HIV-1 seropositivity among the study groups were five individuals (2.5%) from patients group were positive for HIV-1 while, two (1.0%) of the healthy control were positive. The sensitivity and specificity of ELISA test, WB were 100% and 99.8% respectively, the diagnostic accuracy was 97.89%. Moreover, there was a complete agreement between ELISA and RT-PCR and WB results. In addition, there was no statistically significant difference between the two study groups regarding RT-PCR, WB and ELISA results (P>0.05). However there was no statistical significant difference between VL and HIV-1 (P>0.05). We concluded that ELISA was an adequate screening test for the diagnosis of HIV-1 with reasonable sensitivity and specificity. However, it should be confirmed by RT-PCR. RT-PCR was highly sensitive and specific test and was a gold standard test especially important when serological test was borderline. Moreover HIV-1 should be taken into consideration during the follow up of visceral leishmaniasis patients.

Keywords: Visceral leishmaniasis, Seropositivity.

Introduction

Leishmaniasis is a protozoan parasitic disease. The genus leishmania was widely distributed in nature. There are more than 13 species. There are three clinical types of leishmaniasis: cutaneous (Oriental Sore), mucocutaneous (Espundia) and visceral Kala-azar[1]. Visceral leishmaninasis (VL) was caused by leishmania donovani complex (LDC) which includes L. donovani[2]. The new world leishmaniae were carried by sand flies of the genera lutzomyia and pychodopygns while old world one were transmitted by species of genus phlebotomus. New routes of leishmania transmission were discovered, such as sharing of injection, congenital, sexual routes and through blood transfusions[3]. Needle sharing by intravenous drugs users has been proposed as providing an alternative, artificial, and antrhoponotic cycle for leishmania transmission[4]. Seventy percent of VL/HIV co-infected persons in Southern Europe among intravenous drug users suggest that sharing of needles may account co-infection[5]. Studies in Sudan and Kenya have detected L. donovani in domestic animals[6,7]. Leishmaniasis enters the mammalian host during the blood meal of an infected female sand fly. The main complication was severe anemia and loss of weight[8,9]. Patients usually develop the clinical disease after an incubation period varying from a few weeks to several months[10]. A positive reaction may be found in many persons from endemic areas who show no visible skin lesions but have been exposed to infection. Kala-azar the black sickness occurs in the three distinct epidemiologic patterns. The first pattern was dominant in the Mediterranean basin, the Middle East, Southern Russia and parts of China. The reservoir hosts are primarily dogs and foxes[9]. The second pattern prevails in sub-Saharan Africa rodents and small carnivores are the main reservoirs hosts[1]. The third pattern was seen in India and Kenya in which humans appear to be the only reservoir host[11]. The drugs of choice today were the same compounds that were used in the early 1900s. They were extremely toxic and the patients must be monitored closely during treatment[12] .Visceral Leishmaniasis (VL) was the clinical form of leishmaniasis most frequently associated with HIV/AIDS especially in South-Western Europe. The incidence of leishmania/HIV co-infection is increasing in East Africa and the Indian sub-continent[10]. Some researches indicated that HIV can reactivate latent leishmania infection as attacks the host's immune system. Similarly, leishmaniasis seems to up regulate many of the cytokines and growth factors that HIV uses for viral replication and regulation[13]. In Southern Europe, 25-70% of adult VL cases also have HIV infection. The

pentavalent antimonies compounds are the most frequently used drugs in the treatment of VL in HIV positive patients[5].

Methodology

Ethical Consideration: The research was granted ethical approval by Gezira University research ethics committee and Ministry of Health in the Blue Nile State also informed consent was given by participants.

Area and population: This study was conducted at Eldamazin town, the capital of the Blue Nile State which is located 525Km South of Khartoum the capital of Sudan. The State extends from Sinnar State in the North, bordering Ethiopia in the East and the South Sudan into West and South. It is an agricultural and postural State. The population of this State is 861000 persons (census, 2009), most of them are farmers and animal breeders. The State was involved in civil war between North and South of Sudan from 1987 to 2005. As a result of that war there were many people who left their homes and were settled in camps. These conditions led to many health problems including poverty and high incidence of sexually transmitted diseases, such as AIDS in addition to the other endemic diseases in the region like Malaria, Tuberculosis and leishmaniasis. The Blue Nile State is one of the Sudanese leishmaniasis belt areas[14].

Study design: This was an analytic prospective case control study, designed for the seroprevalence of HIV-1 infection among visceral leishmaniasis patients during the period of August 2015 to May 2017.

Study location: This study was performed in Omdurman Military Hospital Department of Microbiology and Eldamazin Hospital Medical Laboratory.

Sample size: The study included adults, males and females known visceral leishmaniasis patients. The total numbers of samples were 200 patients and 200 controls.

Characteristics of Patients group: This group included 200 known visceral leishmaniasis. One hundred fifty three (76.5%) were males and 47(23.5%) were females. The mean age was 21.5 years the youngest was 18 and the oldest was 60 years old.

Characteristics of Control Group: They were (200) healthy persons, 155 (77.5%) were males and 45 (22.5%) were females.

Sample collections: Six mls of blood were taken from patients and controls. Three mls of the blood were put into EDTA containers and the other 3mls were processed as serum samples. The sera were divided into 2 cryotube, stored at –20ºC till used for ELISA and Western Blot. The EDTA blood was stored at -20 ºc and used in the Polymerase Chain Reaction.

Methods: All serum samples were screened by indirect enzymatic-link immunosorbent assay (ELISA) for the detection of anti - HIV-1 antibodies and later the positive samples were tested by WesternBlot (WB) and finally were tested by reverse transcriptase-polymerase chain reaction (RT-PCR) for detection of HIV-1 RNA.

Indirect Enzymatic-Link Immunosorbent Assay (ELISA): Murex HIV-1/2 ELISA was used from Murex Biotech Limited England.

Western Blot Test: Those samples which were positive for anti-HIV-1 were re-examined by western blot kits supplied by Mikrogen Gmbh, Germany.

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR): HIV-1 RT-PCR kit, supplied by Biosewoom Company LTD, Seoul, Korea, was used to examine those samples that were positive for anti-HIV -1.

Statistical analysis: All data were analysis using Statistical Package for Social Sciences (SPSS) soft-ware version (16) USA. We used in data analysis of different variables (ANOVA), Pearson chi-square test and paired samples test.

Results and discussion

Among (200) patients with visceral leishmaniasis, five patients (2.5%) were positive for HIV-1 by both techniques (ELISA and PCR). Moreover, among 200 healthy control individuals three (1.5%) individuals were positive for HIV-1 by ELISA whereas two (1.0%) individuals were positive for HIV-1 by PCR Table (1). The results obtained by Western Blot test (WB) in this study were same as that obtained by PCR for the study groups. However there was no statistical significant difference between visceral leishmaniasis patients and control group ($p > 0.05$). The present study showed no statistically significant difference between PCR and ELISA results ($p>0.05$) as well as between PCR and WB positive and negative results ($p>0.05$) among all studied groups Table-1. Polymerase chain reaction was a valuable method for diagnosis of HIV-1 when the serological test was negative or borderline. This was proved by Tonui[15], they reported a case of HIV type-1 in a 27 years old girl. Serological diagnosis by ELISA was insufficient in making diagnosis of low HIV-1 specific 1gG antibodies in the serum. The prevalence of HIV-1 antibodies in the present study among visceral leishmaniasis patients was 2.5%, while Desjeux[5] in southern Europe, reported that 25-70% of adult visceral leishmaniasis cases also have HIV infection, which was extremely higher than our finding. In East Africa, cases of leishmania/HIV co-infection have been reported in Ethiopia (74), Kenya (15), Malawi (1) and Sudan (3)[16]. Male to female ratio in case of the two studied groups HIV seropositive leishmania patients and HIV sero positive control individuals, was 1.5:1 and 2:1 respectively. This was due mainly to heterosexuality which was main risk factor in Africa Table-2. To evaluate the test performance of the ELISA assay, we used the combined Western Blot/RT PCR results as the reference for calculating sensitivity, specificity and predictive values. RT-PCR and WB tests were used in this study as confirmatory test. The RT-PCR was the most reliable with high sensitivity and specificity. The WB test needs experienced person and may yield indeterminate results.

Table-1: Results of ELISA and RT-PCR of study and control groups:

Groups	ELISA		RT-PCR	
	Positive	Negative	Positive	Negative
Leishmania (n=200)	5 (2.5%)	195 (97.5%)	5 (2.5%)	195 (97.5%)
Control (n=200)	3 (1.5%)	197 (98.5%)	2 (1.0 %)	198 (99%)

Table-2: Mode of transmission of HIV-1 positive in leishmania and control groups.

Mode of transmission	Leishmania	Control	No	%
Heterosexual	4.0	2.0	6.0	75%
Homosexual	00	00	00	00
Blood transfusion	00	1.0	1.0	12.5%
Vertical transmission	00	00	00	00
IVDU	1.0	00	1.0	12.5 %
Total	5.0	3.0	8.0	100%

Conclusion

We concluded that the HIV-1 seropositivity among visceral leishmaniasis (VL) patients was 2.5 %. The molecular biology method in general was superior to serological methods for diagnosis of HIV-1.

Recommendation: We recommended that visceral leishmaniasis patients should be tested for HIV-1. The use of ELISA test for laboratory diagnosis of HIV-1 was highly recommended. The RT- PCR should be used for confirmation of ELISA results.

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