LEISHMANIA/HIV CO-INFECTION
In the Sudan

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﴿وَيَسْأَلُونَكَ عَنِ الرُّوحِ قُلِ الرُّوحُ مِنْ أَمْرِ رَبِّي وَمَا أُوتِيتُم مِّن الْعِمْمِ إِلاَّ قَمِيلاً﴾

الإسراء: 85
To my Parents

With endless

Love
Acknowledgment

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Abstract

Background: The present descriptive cross-sectional study was designed; investigate the prevalence of co-infection of leishmania and immunodeficiency virus (HIV) infection as an emerging problem in Sudan, where the distribution of both infections overlap particularly in the eastern and southern parts of the country.

Methods: In the first component of the study (group 1) a total of 125 serum samples had been confirmed to be positive for HIV during routine screening in National Health Laboratory in Khartoum, were screened for VL using 4 serodiagnostic tests: Direct agglutination test (DAT), Enzyme-linked immunosorbent assay (ELISA), Indirect fluorescent antibody test (IFAT) and Western blot (WB). In second component of the study 185 serum samples from VL suspects (group II) were screened for HIV infection by ELISA, and the reactive samples were confirmed by other two different ELISA techniques

Results: The results of the first component showed that the DAT was positive in 4 (3 %) , ELISA in 37 (30 %) , IFAT in 10 (8 %) and WB in 6 (4.8 %) of the 125 studied samples . 34 (27 %) of the samples were positive by one methods, 8 (6 %) by two methods and only 3 (2.5 % ) by the three methods. None of the samples was positive by the 4 methods. The prevalence of leishmania infection in those HIV individuals was 8.8 (11/125) when only the samples that were positive by two or more serological tests were
considered positive. Nine those individuals were males and only one was a female. Their age ganged between 4 and 50 years. Eight of these cases had AIDS and two were a symptomatic HIV positive individual who had been identified during the routine screening of travelers and blood donors for HIV. The positivity of the different serological tests of VL in those individuals in a descending order was 100 % for ELISA, 54 % for IFAT, 45 % for WB and 27 % for the DAT.

In the second group II the age of the VL suspects ranged between 1 and 50 years. 137 were males and 48 were females. The diagnosis of VL was con by a positive lymph gland smear in 3] (29 %) of the 4 cases. The DAT was positive in 96 (81 %), ELISA in 15-8 (89 %), IFAT in 156 (88 %), the WB in 153 (86 %) and the latex agglutination test for urinary antigens (Katex) in 127 (69 %) of the 185 cases. Screening of those cases for HIV revealed 8 (4%) reactive samples and the HIV infection was confirmed in 4 (2 %) of the samples. All the VL/HIV co-infected cases were males, and their age range between 30 and 40 years. The diagnosis was confirmed parasitologically in one of the four cases, the DAT was positive in two cases, while the ELISA, IFAT, WB, and the Katex were positive in the four (100%) cases.

**Conclusion:** It can be concluded that the leishmania co-infection with HIV is an emerging problem in the Sudan, which requires further studies.
المستخلص

مقدمة: هذا البحث عبارة عن دراسة وصفية مقطعية صممت لتحديد معدل تلازم مرض الكازار والحشوي ومتلازمة نقص المناعة المكتسبة كمشكلة حديثة الظهور في السودان حيث تداخل انتشار المرضى في شرق وجنوب البلاد.

منهجية البحث: المجموعة الأولى عبارة عن 125 عينة بلازمة تأكدت إيجابيتها بالفحص في المعمل القومي بالخرطوم، فحصت نتائجها باستخدام أربعة طرق فحص بلازمة تشخيصية وهي: الدات (DAT)، الاليزا (ELISA)، IFAT، والوسيترن بلوط (WB).

المجموعة الثانية عبارة عن 185 عينة بلازمة أخذت من مرضى متهمين بالكلازار الحشوي، تم فحصها لمكلازار الحشوي في جميع العينات وفحص فيروس نقص المناعة بواسطة الاليزا.

نتائج البحث:

المجموعة الأولى أوضحت ايجابية الدات في 4 (3%) عينة والإليزا في 37 (30%) وIFAT في 10 (8%) والويسترن بلوط في 6 (4.8%) من العينات (125) عينة. وجد أن معدل الكلازار الحشوي في مرضاً نقص المناعة المكتسبة 8.8% (11/125) وذلك باعتبار إيجابية العينات بكافة أربعة الطرق.

المجموعة الثانية أظهرت ايجابية 185 عينة في ثلاث طرق: الاليزا (158 (89%)، وIFAT (156 (88%)، والوسيترن بلوط (153 (86%)، وفحص البول (127 (69% من العينات (185)). بعد تحليل النتائج، تأكد إيجابية 8 (4%) عينات، ونجحت الإيجابية 4 (2%) منها، في الشكل المتكاشف بينهم.

الكلمات الرئيسية: الكازار الحشوي، متلازمة نقص المناعة المكتسبة، الفحص الذي يشمل وسائل تشخيصية مختلفة.
30 - 40 سنة شُخِّص طفل الكلازار من خلال فحص الغدة الليمفاوية في ثلاثة منهم والدات في اثنين منهم بينما الاليزا والهائية والبيسترون بلوط والكاتكس ايجابية في الحالات الأربعة.

الخلاصه: نخلص من هذه الدراسة أن التلازم بين الكلازار الحشوي ومرض نقص المناعة المكتسبة مشكلة وليدة تحتاج لمواجهة ومزيد من البحوث.
Chapter one
Introduction, Literature review & Objective
1.1 Visceral Leishmaniasis

1.1.1 Introduction

The trypansomatid parasite of the genus Leishmania is the etiological agent of a variety of disease manifests, collectively known as leishmaniasis. Leishmaniasis is prevalent throughout the tropical and sub. Tropical regions of Africa, Asia, the Mediterranean, Southern Europe (old world) and south and center of America (new world). Despite enormous efforts, it has proved difficult to predict the exact scale of the impact of the leishmaniasis on public health, since many cases go unreported or misdiagnosed. It is estimated that approximately 12 million people are currently infected and a further 357 million at risk of acquiring leishmaniasis in 88 countries, 72 of which are developing countries and 13 of them are among the least developed in the world. The annual incidence rate is estimated to be 1-1.5 million cases of cutaneous leishmaniasis (CL) and 500,000 cases of visceral leishmaniasis (VL).¹

1.1.1.2. Historical background:

VL. Was first described in 1903 by Leishman and Donovan. Both of these physicians separately, but simultaneously demonstrated parasites in stained smears from the spleen of patients suffering from malaria-like illness, which became known as VL and its causative agent was named Leishmania Donovani.
This is not to say that leishmaniasis did not exist before 1903, on the contrary, Aarchibadi in 1922 described an epidemic of kala-azar which occurred in the Garo hills of Assam in Saudi Arabia as far back as 1870. Cunningham recorded a similar disease that had occurred in 1885, caused by a parasite which was later named leishmania tropica, the causative agent of (CL). Nicolle in 1908 reported that mammals including dogs could act as reservoir hosts of the Leishmania parasite. Swaminath et al in 1942 proved, using human volunteers, that leishmania parasite could be transmitted by the phlebotomus sand flies.¹

1.1.1.3 Epidemiology

1.1.3.1 world wide geographic distributions:
Leishmaniasis is endemic in 82 countries (10 developed and 72 developing, including 13 of the least developed countries) and the World health organization (WHO) estimates incidence of 12 million cases among 350 million at risk and an annual incidence of 600,000 cases.² Leishmaniasis is endemic to all countries of the New World (except Canada, Uruguay and Chile), Mediterranean basin, Middle East, Southwest Asia, India, the newly independent States of former Soviet Central Asia and Transcucasia, East Africa, and China. Despite the wide geographic distribution, human leishmaniasis is often very focal within an endemic area, leading to outbreaks of disease.³
l. 1.1.3.2 Transmission Sites and human Behaviour

Old world VL: in the Mediterranean basin, VL is a zoonosis caused by L. infantum. It occurs in rural areas, in villages, and also in some periurban areas. Most people in this region have doges – the proven reservoir host of L. infantum - as well as other animals that attract sandflies into their houses and therefore favor transmission to humans.

In anthropotic Foci in Bangladesh, India and Nepal the conditions that favor epidemics of Kalazar are known to be rural areas at less than 600 m above sea level, heavy annual rainfall, a mean humidity above 70 %, a maximum temperature of 38 C° and a minimum temperature of 15 C°, with a diurnal variation of less than 7 C°, a abundant vegetation, subsoil water and alluvial soil. Specific risk factors in Kenya are believed to be the proximity of humans to sandfly breeding sites, the hot climate and, finally, the grass-thatched huts that are so easily accessible to sandflies.

Old world Zoonotic CL: The risk of ZCL maybe increased considerably when agricultural projects are launched. Under such circumstances, man-made ecological changes take place and, at the same time, large numbers of non-immune immigrant intrude into an existent sylvatic zoonotic cycle of leishmaniasis. Transmission to humans is favored by the practice of sleeping
outside on terraces without using bed-nets during the hot season, the transmission period.

New World VL: the epidemiology of VL resembles that of the disease in the Mediterranean basin. The habit of keeping dogs and other domestic animals inside the house, particularly during the night, is a risk factor, since dogs attract sandflies. The location of hen-houses close to the main habitation is another important risk factor, as they constitute a major feeding and resting for Lu. Iongipalis, thus increasing the risk of transmission to dogs and humans.

CL and mucocutaneous leishmaniasis, (MCL) in the new world can, for the most part, be considered occupational diseases since they are directly related to professional activities in enzootic areas, especially in forests.

Socioeconomic factors: Primitive housing and low standards of hygiene increase the risk of transmission in peridomestic areas in New World, leishmaniasis is usually predominant in low-income groups.

1.1.3.3 Parasite

Leishmania parasites are protozoa belonging to the subphylum mastigophora which has general features that produced by simple binary fission and possess a kinetoplast. The flagellated forms have a single flagellum. Leishmania parasites can be grown on several
media, NNN, Schneider’s and RPMI media are most commonly used.  

1.1.3.3.1 Life Cycle:
when the female sandfly sucks the blood of an infected human or mammalian host (reservoir), it ingests amastigote forms contained in the blood. The amastigotes pass into the midgut where they transform into promastigotes and multiply by longitudinal binary fission. Four to five days later, the promastigotes move forward to the Oesophagus and Pharynx. When Leishmania promastigotes begin to dog up the oesophageus the feeding sandfly then pumps its oesophageal contents in and out to clear the obstruction, thereby inoculating and thus completing the parasite cycle.  

1.1.1.3.3.2 Classification:
The genus leishmania, as a subdivision of the family of Trypanosatidae; the genus leishmania is again subdivided into the leishmania and Vianna subgenera. The division in subgenera leishmania and Vianna is based on extrinsic characters; there subgeneral are further subdivided in complex based on intrinsic characters (isoenzyme) and further into species: Subgenus is divided into the complex of leishmania (L. donovani (species L.infantum, L. donovani, L. Chagasi, L. archibadi), the L.major complex, the L. tropica complex (species L.tropica and L.Killicki)
, the L. aethiopica complex and the L. Mexicana Complex (with several species). Subgenus Viannia is divided in the L.brazilensis Complex and the L. guyanensis complex, both with several species.\(^5\)

1.1.3.3.3 Identification:

The identification methods make use of both “extrinsic” characters (e.g. clinical manifestations, geographical distribution, epidemiological cycle) and “intrinsic” characters (e.g. size, function, molecular structure). The most widely used of the intrinsic and, more particularly, biochemical methods are analysis of isoenzymes by electrophoresis. In particular, the use of specific DNA probes and monoclonal antibodies has already been shown to be potentially useful for the rapid identification of species without having to isolate parasites in culture.\(^3\)

Genus and species designations for leishmania are arbitrary, because no standard method for species identification has been stated to date.\(^6\) Today we can use many “intrinsic criteria” for isolate classification which may provide more rational bases for species identification.\(^2\)

1.1.3.4 Reservoir Hosts

Broadly speaking, there are two types of leishmaniasis: (1) Zoonotic leishmaniasis, in which the reservoir hosts are wild animals commensals or domestic animals. (2) Anthroponotic
leishmaniasis, in which the reservoir is man. Yet, generalization, such as this should be made with circumspection. CL caused by L. tropica may derive not from human reservoir hosts but from rodents or dogs. Although leishmaniasis caused by L. major is a typically zoonotic, man may constitute a secondary reservoir host in the absence of rodent reservoir hosts, where either the vector is present throughout the year or patients with chronic lesions form a reservoir.  

### 1.1.1.3.5 The Vector

Since the turn of the century scientists have believed the sandfly to be a vector for leishmaniasis, but the transmission of VL and CL via sandflies was not proven until the 1940s. There are six genera: Lutzomyia, Brumptomyia, and Warileya in the New World and Phlebotomus, Sergentomyia, and Chinius in the Old World. Species and subspecies of Phlebotomus and Lutzomyia are the only proven vectors of leishmania, although man-biting flies occur in other genera.  

The majority of female Sandflies require a blood-meal for egg development; however, autogeny (development of eggs without a blood meal) occurs in a few vectors.  

Sandflies are nocturnal feeders but feed during the day if disturbed. They are considered poor fliers, usually not attempting more than a few hundred meters from their breeding locations.
site, with short, difficult flights, even with a slight breeze. Nevertheless, they can be transported surprisingly far by the wind. This restricted flight pattern explains why the transmission of leishmaniasis tends to be focal.6

1.1.1.3.6 Transmission

1.1.1.3.6.1 Transmission Patterns:
Transmission patterns of both CL and VL can be classified as “epidemic” and “endemic”, outbreaks tend to occur either when a group of susceptible hosts move into an area of endemic transmission or when a sandfly habitat is disturbed, as with the establishment of villages or posts in a newly cleared forest.2

1.1.1.3.6.2 Modes of transmission:
Worldwide, vector-borne transmission is the most common mode of transmission of leishmaniasis. Other modes of transmission (Parenteral, Congenital, Sexual, Occupational and person-to-person transmission) may be becoming more relevant in HIV-Positive patients.2

Vector-borne transmission:
When sandflies bite an infected host, they swallow leishmania amastigotes which circulate freely in the host’s blood or inside peripheral blood mononuclear cells such as monocytes. Giemsa staining shows amastigotes, also known as “Leishman-Donovan bodies”, to be 2 x 5 m, oval, and non-flagellated. These
amastigotes migrate to the sandfly’s Proboscis where they develop into stationary, infective-stage organisms that could be qualified as “metacyclic” promastigotes (1.5-4 μm x 14-20 m and monoflagelated). When this infected sandfly bites a second host, e.g., human being, these promastigotes are released and injected along with potent vasodilators (i.e., ‘max adilan’) that produce long-lasting erythema. Macrophages phagocytize these promastigotes and, as it enables them to survive inside the phagolysosome, they turn again into amastigotes. There, they proliferate by binary fission, ultimately causing lysis at the host cells and infection of the surrounding macrophages.

Needle – Sharing:
By March 1993, 18,347 cases of AIDS and about 200 cases of HIV-associated leishmaniasis were detected in Spain of which more than 85 percent occurred among Intravenous drug users (IVDUs).

Alvar described “Cutaneous” leishmania zymodemes in HIV-leishmania co-infected patients which are not found in dogs (MON-24). Furthermore, there is a high variability of L. infantum in drug abusers (MON-1 is the most common zymodeme, but MON-24, MON-28, MON-29, MON-33 and MON-80 have also been found); MON-1 is seen in fewer than one half of 48 sample from co-infected patients.
All these issues may indicate that these zymodemes circulate among drug users who share syringes and, therefore, act reservoirs to a degree that is as yet unknown. In another study Molina et al. tested the indirect xenodiagnosis of VL in 10 HIV-infected patients, of whom nine were IVDUs; they found that minute volumes of blood (0.3 - 0.5 ul) proved infective to Phlebotomus perniciosus, thereby concluding that the possibility of needle-mediated transmission should not be ruled out.

**Transfusion-associated leishmaniasis:**

By July 1995, seven cases of transfusion-associated leishmaniasis had been reported, including four children who received multiple transfusion and three children who received blood from a single donor. Transfusion-associated leishmaniasis requires that the parasites be present in the peripheral blood of the donor, survive processing and storage in the blood bank, and infect the recipient. In endemic areas where the population of potentially infected individuals may be much higher and the screening process for donors less rigorous, transfusion-associated leishmaniasis is probably more common.

**Congenital Transmission**

Several cases of congenital transmission have been reported, for instance when VL was diagnosed in a woman during pregnancy or shortly or shortly after delivery. In addition, L. donovani was
found to traverse the placenta of Syrian hamster and mice. Thus congenital transmission from asymptomatic mothers is considered possible when VL occurs in infants younger than three months.²

**Sexual Transmission:**
Urine and prostatic fluid cultures from patients with VL have yielded promastigotes reports of sexual transmission include transmission from a man to his wife, as well as probable transmission in a homosexual man with AIDS with a rectal lesion who admitted frequent receptive anal intercourse while vacationing in endemic areas of Spain.²

**Laboratory -Acquired:**
By July 1995, nine cases of laboratory- acquired infections had been reported. Six of them were from needle-stick injuries which led to ulcers at the inoculation site; two were related to handling of contaminated specimens; and one to oral exposure, which led to visceral involvement.²

**Person-to Person Transmission:**
Some indirect data, mainly from animal models, suggest that this mode of transmission is theoretically possible via contact with infected fluids (nasal and oral secretions, ‘tonsillopharyngeol mucosa, and urine of patients with VL.²
1.1.1.3.7 Risk Group:
Worldwide, leishmaniasis can still be considered to be a childhood disease; even this, however, is beginning to change. The majority of VL is seen in children younger than five years in areas where transmission involves an animal reservoir, in areas where an animal reservoir has not been reported, CL and VL can be seen in all age groups.²
It has been suggested that men are at higher risk because of occupational exposure and that the access of women to healthcare has been limited due to cultural barriers. Some experimental murine models suggest that women are less likely to develop to clinical symptoms of VL than exposed men. However, this phenomenon” remains unexplained.²
The association of leishmania infection with AIDS has led to a significant shift in the age of people at risk. In the Mediterranean region, the recent shift in the age group prevalence of VL from infants to adult is not due to oscillations in VL incidence but it is seen only where HIV is present and is in part associated with activation of disease in hitherto undetected asymptomatic carries who are also infected with HIV.⁹

1.1.1.4 Clinical forms:
The disease can present itself in man in four different forms, all
with devastating consequences: Cutaneous, diffuse Cutaneous, mucocutaneous, and visceral.

1.1.1.4.1 The Cutaneous and Mucosal Forms:
The Cutaneous forms are the most common (1.0 to 1.5 million cases per year), representing 50-75% of all new cases. CL is known as “little sister” in countries where disease is so common that it is part of the family. It produces skin lesions, sometimes as many as 200 on the face, arms and legs, causing serious disability and permanent scars. Ninety percent of cases occur in Afghanistan, Algeria, Brazil, Iran, Peru, Saudi Arabia and Syria. The Diffuse Cutaneous form is less common, chronic in evolution and especially difficult to treat. It produces lesions resembling leprosy, which do not heal spontaneously. There is systematic relapse after treatment, due to deficiency of the immune response. The mucocutaneous form, also called “espundia” in South America, produces disfiguring lesions to the face, destroying the mucos membranes of the nose, mouth and throat. Most cases of this type are found in Bolivia, Brazil and Peru.

CL of Old World is normally caused by three species of leishmania: L.tropica, L.major and L.aethiopica. New World CL causative agents include L.mexicana, L.peruviana,
L.guyanensis and L.braziliensis simple cutaneous lesions are caused by several other species of leishmania, such as L.amazonensis L.venezulensis and L.inainsoni.

Old World DCL is caused by L.aethiopica. New World DCL has been associated only with members of the L.inexicana complex. MCL is do name correctly applied only to the New World disease known as “espundia” , which is caused by L.baraziliensis orL.panamensis (both species of the subgenus Viannia).³

1.1.1.4.2 VL:
VL (500,000 cases per year), is the most fatal if untreated particularly in cases of co-infection with other diseases , such as AIDS.¹⁰

1. 1.1.4.3 Post Kala-azar dermal leishmaniasis( PKle):
PKDL was first reported from India by BRAHAMA CHARI (1922). It was characterized by the occurrence of skin lesions, macules, papules or nodules, mainly on the face , developing 2-7 years after apparently successful treatment of VL.
PKDL occur mainly in the old world, in particular in India, East Africa and China where L.donovani is the parasite involved, whereas it is rare in areas where VL is caused by L.infantum. Other areas where cases have (PKDL) reported are Kenya , China and Brazil. In Sudan PKDL was recognized in the 19405 by Kirk
and collaborators. In the new world, PKDL occurs sporadically and has been reported after infection with L. Chagasi and L. amazonensis.\textsuperscript{11} 

In India, PKDL is characterized by dermal infiltration with histiocytes, plasma cells, and lymphocytes that is variable in distribution,

1.1.1.5 Pathology of VL: 
Leishmania multiply in cells of the mononuclear phagocyte system, which includes blood monocytes, macrophages, histiocytes, epithelioid cells, Kupffer cells, and reticuloendothelial cells in spleen and lymphoid tissues. Inoculated promastigotes are phagocytosed in the skin and transform into amastigotes and start to divide. One of three events follows: (1) Parasites are killed by a successful immune response and the person becomes immune to reinfection by the species. (2) Local infection is established which persists until the host’s immune response eradicates it or is overwhelmed, permitting dissemination. (3) The infection metastasizes through the blood-stream to the viscera (viscerotropic species), oronasal mucosa (L. braziliensis commonly, others rarely) or skin (especially L. aethiopica and L. mexicana if cell-mediated immunity fails). Distant metastasis via the lymphatics is rare as parasites are destroyed in lymph nodes.
In the endemic situation there are about 30-100 subclinical infection for every case of VL, but fewer in the epidemic situation of Sudan. Risk factors for the development of VL include malnutrition and immune depression due to organ transplantation, hematological nesplania, corticosteroid treatment and HIV infection.¹²

The parasite, L. donovani, gain access to the body through the skin when an infected female sandfly inoculates promastigotes into the dermis. The promastigotes invade local tissue macrophages where they transform into the amastigote form. From the skin the parasites disseminates primarily to the bone marrow, spleen and lymphnodes, but other organs are also involved. In advanced disease, hardly any organ escapes involvement.¹³ In the spleen, the red pulp is heavily infiltrated, and the germinal centers atrophic. Infarcts are common In the liver, Kupffer cells are hypertrophied hyperplastic and heavily parasitized and push out into the sinusoids. In a heavy infection this may reach as for as centrilobular veins. If venous congestion is severe there may be patchy necrosis of groups of hepatocytes, but severe hepatocellular damage is rare. A fibrogenic response may develop, with intralobular proliferation of reticulin fibers and deposition of collagen band S, which may lead to multifocal perisinusoidal fibrosis. Cirrhosis is a rare complication of long-standing VL.
Lymph nodes show sinusoidal cell proliferation and parasitization and depletion of germinal centers. Duodenal and Jejunal mucosa is thickened and villi blunted, the submucosa is infiltrated with parasitized macrophages, plasma cells and lymphocytes. The bone marrow is usually hyperplastic but with some dyserythropoiesis and maturation arrest. Parasites may be found in apparently normal skin and circulate in the blood.¹²

1.1.1.6. Clinical features :

1.1.6.1 Incubation Period :
The clinical incubation period ranges from 3 weeks to over 2 year, but 2-4 months is average. Post primary cases can occur in the immunosuppressed many years after infection.¹²

1. 1.1.6.2 Onset :
In expatriates, and early in epidemics, the onset is usually acute with Sudden appearance of acute fever and rigors in 96 %. In indigenous people in an endemic area the onset is equally commonly insidious, such that the patient may not seek attention for up to a year.¹²

1. 1.1.6.3 Symptoms & Signs:
The table below shows the clinical features of established visceral leishmaniasis.¹²
<table>
<thead>
<tr>
<th>Feature</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>100</td>
</tr>
<tr>
<td>Abdominal</td>
<td>80</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>45</td>
</tr>
<tr>
<td>Cough</td>
<td>76</td>
</tr>
<tr>
<td>Epistaxi</td>
<td>51</td>
</tr>
<tr>
<td>Loss of wight</td>
<td>100</td>
</tr>
<tr>
<td>Oedema</td>
<td>27</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>100</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>75</td>
</tr>
<tr>
<td>Lymphadenopally</td>
<td>5</td>
</tr>
<tr>
<td>India</td>
<td>5</td>
</tr>
<tr>
<td>Africa</td>
<td>84</td>
</tr>
<tr>
<td>Hyperpigmentation</td>
<td></td>
</tr>
<tr>
<td>European</td>
<td>0</td>
</tr>
<tr>
<td>Indians</td>
<td>70</td>
</tr>
</tbody>
</table>

1.1.1.6.4 Differential diagnosis:

The differential diagnosis rests among malaria, brucellosis, bacterial endocarditis, typhoid, miliary tuberculosis and haemopoietic malignancy. Other diseases considered were
infectious hepatitis, amoebic abscess, chronic renal disease, echinococcosis and rheumatic fever.14

1.1.1.6.5 Post Kala-azar manifestations:

All post Kala-azar manifestations are uncommon except PKDL. Some patients develop post Kala-azar mucosal leishmaniasis after or during VL; the parasite involved has been isolated from the nose and shown to be L. donovani. The clinical presentation is indistinguishable from the rare mucosal leishmaniasis caused by L. major, in which the disease is limited to the nasal mucosa without signs of (previous) involvement of other organs. In the eye, several syndromes may be encountered. Post Kala-azar uveitis should be suspected in a patient who complains of impaired vision and red and painful eyes. Moreover post Kala-azar Conjunctivitis and blepharitis, swelling of the conjunctiva and/or eyelids, may occur after recent treatment of VL. Leishmania parasites may be demonstrated in smears17,18. Post Kala-azar larynigitis has been reported from India in the course of PKDL, (1)18 and Post Kala-azar colitis, has been reported in a patient who presented with cholecystitis.19
**1.1.1.6.6 Laboratory findings:**

VL is characterized by pancytopenia, but the severity of changes in each of the three cell lines varies. Causes of anaemia include: Iron deficiency, folate deficiency and haemolysis. Leucopenia in VL is caused by a decrease in neutrophils and there is relatively lymphocytosis. It is unusual to find eosinophils except in concurrent helminthic infection. Platelet count can be in the range of $163 \pm 60 \times 10^9/L$ with count below $50 \times 10^9/L$ in some Patients. The albumin/globulin ratio is inverted and the elevated globulin fraction is $\gamma$-globulin.

Rheumatoid factor and circulating immunocomplexes may be demonstrated; The direct coombs test may be positive. Mild albuminuria may be found in 40 - 70 % of Patients.

**1.1.1.7 Diagnosis:**

The gold standard in each case remains the isolation or identification of the parasite from appropriate tissue.

**1.1.1.7.1 Parasitological methods:**

- **Spleen aspiration:**
  
The most sensitive and the most controversial method, because of feared bleeding complications. As well the severity of infection may be assessed by counting the number of parasites using the grading system developed by Chulay and Bryceson. Higher
sensitivities were reported in Sudan (95-97 %) than in India (71 - 76 %).\textsuperscript{26}

**Bone marrow aspiration:**
A drop of marrow either taken from iliac crest or the sternum is pushed on to a slide, smeared, fixed, with methanol and stained with i Giema’s or leishman’s stain; a high; 85 % sensitivity was reported in China.\textsuperscript{27}

**lymph node aspiration:**
Is by far the easiest and most convenient method and is recommended for use in hospitals and in the field. The inguinal nodes are the most convenient. After cleaning the skin, the node is fixed between 2 fingers and a 20 -22 gauge needle is inserted, and the needle is gently rotated and the fluid is allowed to enter the needle by capillary force and the contents of the needle are expelled by a 2 ml syringe on to a microscope slide and then fixed and stained. The highest sensitivity obtained is 80 %by El-Safi et al.\textsuperscript{28}

**Other body materials:**
In Kenya leishman-Donovani bodies had been demonstrated in some body fluids such as blood, sputum and urine of some patients.\textsuperscript{29}
Culture and animal inoculation

Materials obtained by lymph node, bone marrow or spleen aspiration may be used for culture. NNN, schneider’s and RPMI are the most commonly used media. The highest obtained sensitivity is 92% by culture of venous blood centrifuges. Animal inoculation may be useful, especially when contamination is expected. Rhors reported a sensitivity of 45% for animal inoculation.

1.1.1.7.2 Serological Methods

Their main asset is non-invasiveness, but they all have restrictions in common. Differentiation between clinical and subclinical or past infection is not possible. Titers may remain high for years and cannot be used for evaluation of therapy. Cross reactions with other pathogens, e.g. trypanosomes, may occur depending on geographical location. Some serological techniques need expensive, sophisticated equipment which precludes their used in remote areas.

Direct agglutination test (DAT):

DAT uses promastigotes stained with coomassie blue as antigen and mercaptoethanol is used to increase sensitivity and specificity. The test can be read by eye, large number of tests can be done simultaneously and it is suitable for field conditions. The test usually takes 48 h, but this may be shortened to 24 h under certain
Positive test results may be obtained with patients with active VL, subclinical infection, and those who are infected more than 7 years previously. Specificity of 98.8% and sensitivity of 100 were recorded. However cross-reaction, unsuitability of the antigen and subjectivity of test result while replication was repeatedly documented; are the major disadvantages of DAT.

**Enzyme-linked immunosorbent assay (ELISA)**

The standard ELISA uses soluble or whole promastigote antigen and the result is read by measuring optical density. Polystyrene surface layer of micrometer plates sensitised with soluble leishmania promastigote antigen resulted in satisfactory test performance for detection of specific antibodies in Mediterranean VL patients.

A recombinant antigen rK39-ELISA has been adapted to an immunochromatographic strip test, which showed 100% sensitivity and 98% specificity in a study in India. In a study in Sudan, rK 39 ELISA proved more sensitive than the DAT in diagnosis of VL 93% and 80% respectively.

**Indirect immunofluorescence test (IFAT)**

Requires an immunofluorescence microscope and is therefore unsuitable for use in the field. The minute amount of leishmania antigen required, and stability of the acetone-fixed antigen preparation rendered applicability of IFAT rather feasible at
moderately equipped laboratories. The use of homologous L.chagasi strain in IFAT had resulted in a higher sensitivity for diagnosis of VL in Brazil as compared to the heterologous isolate of L.mexicana.38

**Immunoblotting (Western Blot, WB):**
The performance of leishmania soluble antigens for VL diagnosis was improved through electrophoresis separation of individual reactive epitopes as in immunobloting techniques.39 It has the capability to differentiate Kala-azar from lymphoprolifertive and autoimmune diseases that could not be differentiated by ELISA and IFAT.40

**Latex agglutination test (LAT) for Serum:**
Leishmania antigens are adsorbed to latex particles and agglutination occur after addition of serum that contain antileishmania antibodies. It is a quick simple and feasible serodiagnostic test. De korte et al performed LAT for detection of anti-L.infantum antibodies in canine VL reservoir , but they obtained a lower sensitivity for LAT in comparison to DAT.41

**Complement fixation test (CFT):**
A small amount of the tested serum mixed with leishmania antigen and some of the complement . Then the a sensitized sheep Red
cells is added if the serum contain antileishmania antibodies, antigen-antibody reaction occur and the complement would be fixed before the addition of the sensitized Red cells, so no haemolysis will take place indicating the positivity of the test. In one study CFT was reported as a highly sensitive test for south American VL.  

Non-specific serological diagnostic test:

Formol gel test (FGT):

Napiers described it for the first time in 1922. It depends on the increase of immunoglobulins level in patients with active kala-azar.  

FGT is performed by mixing one ml of serum with tow drops of formulin 40% and allowed to stand for up to 30 minutes. Whitening and Jellying of the serum usually take place within five minutes if not immediately. A milky appearance without jellification of the serum was thought to occur in patients with early or subclinical VL infection.  

1.1.1.7.3 Tests of cellular immunity:

Leishmanin skin test (LST) or Montenegro test

Measures delayed-type hypersensitivity of the individual in terms of induration of skin in reaction to an intradermal injection of Leishmania antigen, usually a suspension of promastigotes or a soluble friction. A value of > 5 mm is considered positive. It was found to be useful indifferentiating between active and treated cases of VL as in the activedisease cellular mediated response is
diminished}; LST is positive in relapsed cases of VL cases with concomitant PKDL or mucosal leishmaniasis, also it is positive in active and past infection of cutaneous leishmaniasis for life.\textsuperscript{12,46,47} LST is recommended as simple, effective and useful epidemiological field tool that could determine the distribution rates of leishmania infection.\textsuperscript{48}

**Lymphocyte proliferation test (LAT):**

Peripheral blood lymphocytes of sensitized (exposed) individuals, when exposed to antigens of leishmania in vitro, undergo division which can be measured by incorporation of radio-labeled thymidine into the DNA of dividing cells. It has been reported that in active VL cases, no proliferation is found after stimulation with leishmania antigen and this defect is specific for leishmania antigen.\textsuperscript{20}

**1.1.1.7.4 Molecular methods:**

**Polymerase chain reaction (PCR):**

DNA-PCR uses single-stranded DNA as a template for the synthesis of a complementary new strand; what is called DNA amplification. PCR is a promising tool in the identification and detection of DNA of L. donovani in lymph nodes, spleen and blood.\textsuperscript{49,50}

**1.1.1.8 Treatment:**

**General considerations:**
Intercurrent infections should be actively sought and treated; ferrous sulfate and folic acid may be given in cases of severe anemia. It is of great importance to ensure adequate nutrition. Blood transfusion should be given only to those who are in heart failure.

**Pentavalent antimony (pentostam):**

Acts against leishmania in anumber of ways including inhibition of Purine nucleotide triphosphate and macromolecular synthesis. In a review of 1593 VL cases treated; 98 % were reported to have responded well to treatment.

**Pentamidine:**

Acts on the kinetoplast DNA-mitochondrial complex, given as a slow intramuscular injection; doses of 2-4 mg/kg are given 1-3 times per week until clinical and parasitological response in noted. It is more toxic and should be given only in hospital. Cure rates of 82 % with different diamidines is achieved.

**Amphotericin B. (AME):**

Acts on sterols and phospholipides in cell membranes and is widely used as a fungostatic agent. In a study in India, a low dose and short regimen of 0.5 mg/kg on alternate days. for a total of 14 doses gave excellent results, with a cure rate of 100 % and no serious side effects.

**Liposomal amphotericin B (L-AMB**

Leishmania is an intracellular parasite of macrophages and macrophages clear liposomal particles from the circulation, there
for concentrate the efficacy of conventional AMB especially in the macrophages without the toxic effects of circulating drug.

**Aminosidine**

Is an aminoglycoside antibiotic with antileishmanial activity in vitro and in vivo. Given as a single drug to newly diagnosed casesaminosidine cured 15 of 19 patients in Kenya after 19 injections.  

**Allopurinol:**

Its action may be to inhibit parasite adenylosuccinate synthetase or adenine phosphoribosyltransferase.  

**Interferon- y (IFN- x y):**

In a study in Barzil, 6 of 8 VL patients unresponsive to antimony alone responded to combined treatment with interferon y and stibogluconate as did 8 of 9 patients with previously untreated disease.  

**Imidazole Compounds:**

These drugs act on sterol metabolism. Fluconazole was tested in India and found to produce apparent cure in 8 out of 11 patients, all of whom relapsed.  

**Treatment of Relapse**

In cases of drug resistance, L-AMB was shown to be effective.
1.1.1.9 Prevention of Leishmania infection:

For the individual traveler, there is no effective chemoprophylaxis or immunoprophylaxis to prevent infection. Vaccines continue to be evaluated, but none are currently useful. Efforts to decrease the canine reservoir where present by elimination of feral dogs and screening (serology and physical examination) of domestic dogs can lead to a decrease in the number of cases of human VL.²

Control of the sandfly populations is presumed to be an important aspect of control programs, but there are few well-documented examples of interventions leading to a decrease in the disease. Knowledge of a vector’s flying, breeding, and resting habits can lead to focused insecticide use, thus reducing exposure and expense. Control strategies can include any of these points, but in practice, an integrated approach should be used that includes interventions designed to decrease the number of reservoir hosts and sandfly vectors and to decrease contact of humans with sandflies.⁶

Leishmaniasis is closely related to HIV infection in those areas where both diseases are endemic. Thus, clear and updated universal sanitary policies against AIDS are required from governments and public institutions, especially for the poorest groups of people who have fewer resources.

1.1.2 Human Immunodeficiency Virus

1.1.2.1 Historical Background:
In 1981 the Centers for Disease Control CDC published two reports describing cases of Pneumocystis carinii pneumonia and Kaposi’s sarcoma in previously healthy, young male homosexual. Pneumocystis carinii pneumonia until then was mainly known to occur in immunodepressed patients after organ transplantation, after cancer chemotherapy, or in genetic immunodeficiency states. It was soon recognized that these male homosexuals had severe immunodeficiencies with a profound depression of so called Thelper lymphocytes. Shortly after, the same kind of immunodeficiency state was recognized in intravenous drug users (IVDUs), transfusion recipient, hemophiles, and newborn infants of IV drug dependent mothers. In late 1982 the name acquired immunodeficiency syndrome (AIDS) was coined for the syndrome. The virus was initially named lymphadenopathy-associated virus (LAV) by the French group, human T lymphotropic virus type III (HTLV-IH) by Gallo and colleagues (American group) and AIDS-related virus (ARV) by Levy and coworkers. In 1986 a unifying nomenclature for immunodeficiency viruses was abbreviated HIV. In 1986, a second immunodeficiency virus was identified in West Africa, which also causes AIDS but does not cross-react serologically with HIV in screening tests. It therefore was designated HIV-2, and the first HIV was subsequently renamed HIV-1.

1.1.2.2 Epidemiology:

The epidemic of human immunodeficiency virus (PHV) infection and AIDS emerged in the last quarter of the 20th century, and within less than two decades has affected over 190 countries.

1.1.2.2.1 Types of HIV:
HIV infection can be caused by HIV-1 and HIV-2, the two main types of the virus. Globally, HIV-1 accounts for most HIV infections, and HIV-2 appears largely confined to West Africa, with foci in Angola and Mozambique and some cases have been reported in Europe, the Americans, and India. Compared with HIV-1, HIV-2 appears less transmissible through sexual intercourse, its spread is slower, and the disease it causes progresses more slowly. In contrast to HIV-1, HIV-2 prevalence increases steadily with age.59

1.1.2.2.2 Modes of transmission:

HIV can be transmitted in three ways: through sexual intercourse, through blood, and from mother to child.59

Sexual transmission:

Accounts for about three fourth of all HIV infections. Worldwide, more than 90% of HIV infections are associated with heterosexual transmission.60

Vertical transmission:

Mother-to-child transmission of HIV includes transmission during pregnancy, during delivery and through breast-feeding. Data from various countries suggest that as many as 15% of babies breast-fed by HIV-infected mothers may become infected through breast-feeding.59

Blood and blood products:
The risk of acquiring HIV infection through blood transfusion has varied by region, depending on the area’s seroprevalence among blood donors, and the sensitivity of the screening strategies. Furthermore, three recipients of factor VIII concentrates for treatment of hemophilia were reported to have developed P. carinii pneumonia, which suggested that transfusion of blood and blood products was a plausible mechanism for transmission.60

**Allograft and organ transplantation:**

Transmission of HIV through allograft transplantation has been uncommon and represents a minute proportion of all AIDS cases.60

**Injection drug users:**

IDU-associated AIDS cases constitute the second largest risk exposure category in CDC surveillance statistics.60

**HIV-contaminated instruments:**

In resource-poor areas of Africa, Asia, and Latin America, the potential exists for transmission of HIV through unsanitary use of needles. Concerning occupational exposure, nurses are most commonly exposed.60

**1.1.2.2.3 Risk group:**

Homosexuals, the main risk factors for HIV infection as determined by cohort studies are anal intercourse, particularly receptive, and high numbers of partners. Bisexual men may have been at special risk and served as a bridge population for the human HIV in to female population.60
Heterosexuals: Risk factors associated with HIV infection in heterosexuals include number of sexual partners, sex partner of an infected person.

Injecting drug users (IDUs): IDU-associated AIDS cases constitutes the second largest risk exposure category in CDC surveillance statistics.\(^3\)

Moreover, high-risk sexual practices and drug use are related behaviors.\(^61\)

Blood transfusion: the risk of acquiring HIV infection through blood transfusion has varied by region, depending on the areas seroprevalence among blood donors, and the sensitivity of the screening strategies.\(^60\)

1.1.2.2.4 Infectiousness:

The viral load may be the key unifying features of transmission risk.\(^60\) So, in vertical transmission, transmission rates appear to be strongly influenced by the level of maternal viremia, with higher levels producing higher infectiousness.\(^59\) Higher transmission rates have been associated with depleted CD4 +ve cells among HIV-infected sexual partners.\(^60\) Genital ulcers and some nonulcerative STDs, such as gonorrheal and chlamydia infections, facilitate transmission of HIV by increasing the infectiousness of infected individuals and the susceptibility to HIV of noninfected sex partners.\(^59\)

1.1.2.3 Virology: Genome and virion structure:

Retroviruses are enveloped, single-strand RNA virus that rely on a unique enzyme, reverse transcriptase, to convert their RNA
genome to a DNA “provirus”, which is integrated into the cellular genome. The viral envelope is a lipid bilayer that is produced by the cellular plasma membrane and contains the producing viral Env glycoprotein. The core Viral particle is composed of P24 capsid (CA) protein and contains the viral RNA and enzymes. All retroviruses have in common the three coding regions gag, pol, and env, which encode the capsid proteins (Gag), the viral enzymes necessary for replication (Po1), and the external glycoprotein (Env) that protrudes out of the lipid viral envelope and is responsible for the infectivity of the viral particle by means of attachment to specific cellular receptors. The viral enzymes encoded by pol. are reverse transcriptase, integrase and protease. The additional proteins expressed by HIV-1 are part of the viral particle (i.e., Vif, Vpr, Vpx), regulate directly viral gene expression (i.e., Tat, Rev), or interact with the cellular machinery to promote virus propagation (i.e., VPU,Nef).62

1.1.2.4 Pathology:

The CD4 molecule is the principal cell surface receptor for HIV entry in to target cells. The CD4 molecule is expressed not only on T helper cells but also on other cell types,63 including monocytes and macrophages, the langerhans cells of the skin and dendritic cells; cells in the brain such as macrophages, microglial cells, astrocytes, and endothelial cells, B lymphocytes, cells of the intestinal mucosa; and cells of the cervical endothelium.58

1.1.2.4.1 Mechanisms of CD4+ T-cell Depletion and Dysfusion:

These can? be divided into two groups; direct virologic mechanisms that result from an HIV-mediated cytopathic effect, and indirect, nonvirologic phenomena triggered during the course
of HIV infection. With regard to direct virologic mechanisms, HIV-mediated cytopathic effects may occur through their single-cell killing or HIV-induced syncytia formation. The group of indirect nonvirologic mechanisms, anergy, superantigens, apoptosis, and virus-specific immune responses. HIV-specific immune responses, including humoral and cell-mediated immunity, play an important role in the control of HIV replication and spread in vivo. These phenomena may contribute significantly to the elimination of HIV-infected cells, including CD4+ T-cells, macrophages, and follicular dendritic cells (FDCs) or by antibody-dependent cellular cytotoxicity. It has been hypothesized that HIV may cause immunosuppression and disease progression by virus-specific cytotoxic T cell-mediated immunopathology.64

1.1.2.4.2 The Course of HIV Infection:

On the basis of the duration of HIV infection and the kinetics of virologic and immunologic events observed throughout HIV disease, three dominant patterns of evolution of HIV disease have been described: (1) 8% to 90% of PHV-infected persons are “typical progressors” and experience a course of HIV disease with a mediansurvival time of approximately 10 years; (2) 5% to 10% of HIV-infected persons are “rapid progressors” and experience an unusual rapid (3 to 4 years) course of HIV disease; (3) about 5% of HIV-infected persons do not experience disease progression for an extended period of time (at least 7 years) and are termed “long-term nonprogressors” (LTNP).64

1.1.2.4.2.1 Typical Progressors:

The typical course of HIV infection includes three phases:
Primary infection, clinical latency, and clinically apparent disease. The diagnosis of primary HIV infection is made in only a minor percentage of cases. Difficulties reside in the variable severity and nonspecificity of the clinical syndrome, which is characterized by mononucleosis-or flu-like symptoms such as fever, lethargy, sore throat, malaise, maculopapular rash, lymphadenopathy, arthralgias, myalgias, headaches, retroorbital pain, photophobia, and, rarely, meningitis. The phase of primary infection is followed by the long, clinically latent period of HIV infection. In typical progressors, this phase of infection may last for years (median, 8 to 10 years). The advanced phase of infection, is characterized by severe and persistent constitutional signs and symptoms or by opportunistic infections or neoplasms, or both.

1.1.2.4.2.2 Rapid Progressors:

In a minor percentage (5% to 10%) of HIV-infected persons, rapid progression to AIDS occurs within 2 to 3 years after seroconversion, the immune responses are usually defective in these rapid progressors; the level of antibodies against HIV proteins and neutralizing antibody are low or absent, and the CD8+ T cell-mediated suppression of HIV replication is severely impaired.

1.1.2.4.2.3 Long-Term Nonprogressors (LTNPs):

It has become clear that a small percentage of infected persons (5%) do not experience clinical progression of HIV infection and have stable CD4+ T-cell counts for many years (7 or more years) despite lack of therapy. From an immunologic standpoint, immune functions are conserved in LTNPs, and both HIV-specific humoral and cell-mediated immune responses are very strong. Cytokines
that induce HIV expression include IL-1, IL-2, IL-3, IL-6, IL-12, granulocyte-macrophage colony-stimulating factor (M-CSF), TNF-a, and TNF-B. Of these, IL-2 and IL-12 induce virus expression only in T cells, TNF-a and TNF-B induce expression in both T cells and macrophages, and the remaining cytokines induce expression only in macrophages.\textsuperscript{64}

1.1.2.4.3 Induction of HIV Expression:

Several factors have been proposed as being involved in the regulation of HIV expression and potentiation and modulation of virus replications. These include both Tat and Rev are crucial for efficient virus replication, as well, it has been demonstrated that certain cellular transcription factor that bind to consensus sequences on the proviral long terminal repeat (LTR), including Spl, TATA, TAR, and NF-KB, activate HIV RNA synthesis and thus exert positive regulation on viral transcription.\textsuperscript{64}

Another is the state of immune activation, as in CD4+ T cells, efficient virus replication in monocytes or macrophages on the state of cellular activation and differentiation, moreover several pathogens including cytomegalovirus, herpes simplex virus, hepatitis B virus, human herpesvirus 6, human T-cell lymphotropic virus type I, and microbes such as mycoplasma, have been shown to enhance HIV expression in several experimental in vitro systems.\textsuperscript{66}

1.1.2.5 Immune Response to HIV:

The immune response to the HIV is determined by many complex factors. First, the extraordinary host-virus interactions that lead to the pathogenesis of AIDS induce profound functional host immune
defects, beginning soon after infection with HIV. Prominent forms of HIV-induced immune dysfuction include defects in T- and B-cell responses to specific antigens, polyclonal hypergammaglobulinaemia, enhanced autoantibody and immune complex formation, dysregulated cytokine production, decreased natural killer cell activity, and defective monocyte and dendritic cell function. Second, evidence suggests that the route of HIV infection, the amount of HIV in the inoculum, the pathogenic potential of a given HIV strain, and host genetic factors may modify the host response to HIV. Third, evidence suggest that some components of an immune response to HIV may enhance HIV infectivity or may be directly responsible for clinical manifestations of the disease. Fourth, the remarkable ability of HIV to mutate genome sequences and change the primary amino acid sequence of HIV proteins effectively allows HIV to evade otherwise effective antiviral immune responses.

1.1.2.5.1 Antibody Response to HIV:

Neutralizing (anti-IHV) antibodies inhibit the infectivity of free HIV or HIV-infected cells and have been proposed to be one component of a salutary or protective anti-HIV immune response. The epitopes to which most anti-HIV antibodies bind have been located on envelope glycoproteins gp120 or gp 41. Another type of antibodies are ADCC antibodies like anti-gp 160 antibodies in the serum and cerebrospinal fluid of HIV-infected persons bind to IgG Fc receptors (R)-bearing natural killer (NK) cells by means of the antibody Fc region and sensitize IgG FcR-positive cells to kill HIV gp 160-expressing, or gp120-coated, target cells. Moreover anti-p24 antibodies appear within the first 2 weeks of acute HIV infection and rise to their highest levels during the asymptomatic
seropositive stage and then fall to usually undetectable levels with the onset of AIDS. Antibodies to HIV Rev, Nef, Tat, Vpu, Vpr and HIV protease proteins have been reported in various percentages of HIV proteins. Robinson and colleagues described antibodies in AIDS patient sera that augment rather than inhibit HIV infectivity in vitro. These “HIV-enhancing” antibodies bind to epitopes of Env gp 41.68

1.1.2.5.2 T-lymphocyte Response to HIV:

Several MHC class II-restricted helper T-cell epitopes of HIV proteins are recognized by HIV-infected humans and immunized animals. These helper T-cell epitopes have been found in many HIV proteins, including Env, Gag, and Pol. As well MHC class I-restricted CTLs have been demonstrated against Gag, Env, Nef and Pol HIV proteins. In primary HIV infection, the initial fall in viremia correlates best with the appearance in peripheral blood of anti HIV MHC class I- restricted CD8+ CTLS.68

1.1.2.5.3 Non-T-cell mediated cellular immune response:

In the other hand, non-T-cell mediated cellular immune response, as is mediated by NK and other FCR-positive cells that directly kill virally infected cells or that mediate ADCC, is potentially important as an anti-HIV immune response, because these forms of immunity can eliminate virally infected cells in a non MHC-restricted fashion and do not require a memory T-cell response for effector cell induction. Monocytes from a symptomatic seropositive persons mediate ADCC against HIV-coated target cells, and mediate monocyte tumoricidal activity in vitro- a potential mechanism of immune response against Kaposi’s sarcoma and other tumors that occur in AIDS.68
1.1.2.5.4 Pathogenic Versus Salutary Anti-HIV Immune Response:

HIV-specific antibody response and anti-HIV CTL responses decrease in the wake of progression to AIDS suggests that these immune response promote the symptomatic HIV seropositive state, on the other hand, the presence of anti-HIV CTLs capable of killing a variety of types of HIV-infected antigen-presenting cells in lymph nodes, bone marrow, and thymus support to the notion that, anti-HIV CTLs that originally keep HIV infection in check by killing virally infected cells, by continued killing of antigen-presenting cells and other immune types could gradually promote progressive immune system dysfunction. Moreover the presence of enhancing antibody against HIV Env gp 41 has been associated with progressive HIV infection.68

1.1.2.6. Clinical Spectrum of HIV Disease:

1.1.2.6.1 Stages:

A reasonable approach to categorizing HIV disease is to divide the stage of illness into six categories based on a combination of clinical features and CD4 counts.69

1.1.2.6.1.1 Initial infection: Acute seroconversion Syndrome:

Although initial infection with HIV may be asymptomatic, as many as 50% of individuals with acute infection report symptoms
of a flu-like or mononucleosis-like illness. The most common symptom reported is fever, esophagitis, myalgia, arthralgia, diarrhoea, nausea, vomiting, and headache observed less frequently.\textsuperscript{69}

\textbf{1.1.2.6.1.2. Early HIV disease:}

Early stage HIV disease is defined by a CD4 cell count greater than 500 cells/mm.

Dermatologic abnormalities are the most common HIV-associated findings among patients with early HIV disease.\textsuperscript{69}

\textbf{1.1.2.6.1.3 Intermediate stage of HIV disease:}

The intermediate stage of HIV disease is defined by a CD4 count between 200 and 500 cells/mm. Although the relative risk of developing new opportunistic infections is higher among patients with intermediate-stage disease than early disease, most patients with intermediate-stage disease remain asymptomatic or demonstrate only mild disease manifestation.\textsuperscript{69}

\textbf{1.2.6.1.4 Late-stage disease:}

Late-stage HIV disease is defined by a CD4 cell count between 50 and 200 cells/mm. Based on the new CDC classification system, all patients with CD4 counts less than 200 cells/mm are categorized as having AIDS. This change in the classification system was based on natural history cohort data that indicated the risk of developing a new AIDS-defining condition rises dramatically when CD4 counts drop below 200 cells/mm. The most common opportunistic infections to affect this group of patients is (PCP) (Pneumocystis carinii Pneumonia).
Patients with late-stage disease are also at substantial risk of developing other opportunistic infections, including Toxoplasma gondii infection, encephalitis, cryptosporidiosis, isoporoiasis, tuberculosis, B-cell lymphoma, kaposi’s sarcoma, and esophageal candidiasis. (3)

1.1.2.6.1.5 Advanced HIV disease:

Advanced HIV disease is defined as a CD4 count of less than 50 cells/mm. The risk of developing certain opportunistic infections that are associated with more profound immunosuppression becomes significantly higher when the CD4 counts drop below 50 cells/mm. A number of patients with advanced HIV infection develop significant, involuntary weight loss, often referred to as the HIV wasting syndrome, patients who lose more than 10 lb of their usual body weight, with no obvious explanation, are diagnosed as having the syndrome. Disorders of the brain become especially common in advanced HIV disease. (69)

1.1.2.6.1.6. Terminal HIV disease:

From a medical standpoint, the diagnosis of terminal-stage HIV disease is usually based on an inability to control the symptoms of disease, because no treatments are available for the particular disorder or, more commonly, because available treatments become ineffective. In such instances, it is appropriate for treating physicians to make the transition from one of providing primary provision of comfort. This includes psychological support, family support, and aggressive pain managements. (69)

1.1.2.6.2 Opportunistic tumors:
The emergence of NHL (Non Hodgkin Lymphoma) and Kaposi’s sarcoma as the two most common and characteristic malignancies of AIDS was not surprising, because the association of with various other states of congenital and acquired immune deficiency had been documented for several decades. The most frequent opportunistic tumor, Kaposi’s sarcoma, is observed in about 20% of patients with AIDS. On the other hand malignant lymphomas of HIV-infected patients differ from other known lymphomas by their localization, degree of malignancy, and response to therapy.

1.1.2.7 Diagnosis of HIV:

1.1.2.7.1 Antibody Detection:

1.1.2.7.1.1 Preliminary Screening Tests:

- Enzyme linked immunoassay (ELISA):

ELISA test is the standard screening test for HIV infection. Individuals with indeterminate or positive ELISA results should undergo confirmation testing (usually with Western blot testing) to determine if the reactivity is secondary to HIV infection or secondary to cross-reacting antibodies. Most of the commercially marketed ELISA preparations have had a sensitivity of at least 99.5% and specificity of greater than 99.8% in large-scale testing of individuals with high-risk behavior for HIV acquisition.

- Simple rapid tests:

1. Rapid latex agglutination assays: a procedure that can be performed within a matter of a few minutes and that requires a minimum of reagents and technical skills.
2. Passive hemagglutination assay: one example is the autologous red cell agglutination assay developed by Kemp and colleagues.

3. Dot-blot immunoassay: using this method to screen sera for high-risk patients, one group reported a 93% concordance between results obtained by dot-blot and by ELISA.\textsuperscript{71}

1.1.2.7.1.2 Confirmatory tests:

- Western blot: in the United States and elsewhere, this confirmatory role most frequently involves the use of an HIV specific Western blot, of which numerous commercial preparations have been marketed. Although estimates for the sensitivity and specificity of HIV Western blot vary somewhat among manufacturers, comparative surveys have shown that most preparations afford a sensitivity of at least 96%.\textsuperscript{71}

- Indirect immunofluorescence assay (IFA): although the performance time of commercially available Western blot kits is in the range of a few hours, some laboratories prefer to use the FAD-licensed indirect immunofluorescence assay (IFA) for screening or to substitute it for the conventional immunoblot as a confirmatory assay.\textsuperscript{71}

- Radioimmuno precipitation assay (RIPA): an alternative test that is sometimes used as a confirmatory assay over the conventional Western blot is the RIPA. However, its use is largely restricted to laboratories that have the facilities and expertise to propagate HIV in continuous cell culture.\textsuperscript{72}

- Polymerase chain reaction (PCR): PCR methods enable the amplification of discrete fragments of the viral genome. This may be performed for diagnostic purposes (i.e., as a confirmatory assay
to supplement serologic diagnosis) or for research purposes to facilitate isolation and study of select portions of the viral genome.\textsuperscript{72}

1.1.2.7.2 Antigen detection (P24 antigen capture assay):

Although the level of serum P24 antigen may vary from individual to individual, this antigen can be detected relatively early after HIV exposure in many patients, and detection often precedes the process of seroconversion by several weeks.\textsuperscript{73}

1.1.2.7.3 Viral isolation:

Isolation of HIV can be performed using plasma or peripheral blood mononuclear cells from the peripheral blood or lymphoid tissues of the infected patients.\textsuperscript{71}

1.1.2.7.4 Detection of viral nucleic acids:

1.1.2.7.4.1 PCR:

DNA-PCR is a method for detecting and amplifying proviral DNAs from the cells of an infected host. DNA-PCR is frequently employed for the early detection of HIV infection or to help resolve inconsistent findings obtained by the HIV ELISA and immunoblotting.\textsuperscript{71}

1.1.2.7.4.2 Branched DNA (bDNA) assay:

This method differs fundamentally from PCR in that it relies on quantitation of HIV RNA by signal amplification rather than the target amplification as used in PCR. The bDNA assay exhibits good interassay reproducibility, is comparatively easy to perform, and has already been shown to be useful in monitoring changes in
viral levels over time or monitoring responses to therapeutic interventions.  

1.2 objectives: 

1- To determine the prevalence of HIV infection among VL suspects from highly endemic area (in Eastern Sudan). 

2- To study the prevalence of leishmania infection among HIV confirmed individuals.
1.2.1 Literature Review

1.2.1.1 Visceral Leishmaniasis in Sudan

1.2.1.1.1 History and Epidemiology:

1.2.1.1.1 Distribution:

One year after the first cases of Kala-azar in Africa was diagnosed by Laveran in 1903, Kala-azar was diagnosed for the first time in the Sudan in an 8 - years old by from Meshra-er-Reg, in Bahr-el- Ghazal district , Southern Sudan, by Neave. In subsequent years several tens of cases, including 2 British officials”, were reported from the east of the country from about Sennar to Kassala and the Ethiopian border; introduction from Abyssinia was considered. The Situation caused so much concern that first (1909, under Bousfield) and second (1909 - 1913, under Thomson and Marshall ) Kala-azar commissions were appointed by the Sudanese Government.

Extensive investigations were done in Kassala and Blue Nile districts and a map of the distribution of the disease was drawn. In the final report of the second Kala-azar commission, it was concluded that in Sennar province mostly children with average age of about 12 years were affected, whereas in Kassala province adults were commonly affected; the disease ran an acute course and chronic cases were not met with; the disease was not invariably fatal and recovery was possible. New, small foci were identified, such as Kapoeta in Equatoria Province in the South and Darfur Province in the west. Sporadic cases were reported from Kordofan, originating from Talodi, Um-Ruaba and Um-Talha; One case occurred in 1922 in a village situated on the Blue Nile near
Kharotum. As indicated before by Thomson, occurrence along Khors (water courses) and steam and in the period after the rains, about October (the “Darrad”) was observed.

Further spread eastward to the Ethiopian border occurred. In the 1950’s, Blue Nile Province remained the main endemic area and an upward trend in the number of cases was noted; the distribution, however remained patchy, with Singa as the main focus in Blue Nile Province, Kapoeta in Equatoria and Gedaref in Kassala Province.

In Sudan, Kala-azar was reported mainly among rural populations inhabiting villages and small towns with poor hygiene conditions.

The disease was more dominant among adults than in children. Kalajar outbreaks in Sudan could be attributed to the presence of some individuals with PKDL, the movement of non-immune population to endemic areas and the movement of nomads from north to South that introduced the disease to new areas.

1.2.1.1.2 Parasite:

Using PCR analysis proved that L. donovani causes VL, PKDL and MCL in eastern Sudan. L. donovani in Sudan has a predominant genome variants; MON 18, MON 30, MON 81, MON 82. 50, 83, 88

1.2.1.1.3 The Vector:

The fly was identified as phlebotomus (P) langeroni, but it was probably var. orientalis. P. orientalis was found in Gedaref State and in the Diner National Park. It has been reported that P.orientalis was active from April to June, and its population
increases during the dry Season and their numbers fall sharply in the rainy Season. Sandflies were strictly confined to Acacia Seyal and Balanites aegyptiCa forests and to the villages in or near these forests.\(^{(4)}\)\(^{92}\) In the Kapoeta focus, P. martina was thought to be the vector as is assumed for VL in Kenya.\(^{28}\)

1.2.1.1.4 The Reservoir:

It has been confirmed that the infection of a Nile Rat by L. donovani using PCR analysis for spleen specimens, in a focus of endemic Kala-azar in Southeastern Sudan, at the borders with Ethiopia.\(^{50}\) Moreover the anti-L. donovani antibodies were detected in donkeys, cows, goats and K the Nile Rat.\(^{93}\) It has been reported that, three rodents species and two canine species were found to be naturally infected with L. donovani in Palioch-Malakal area in Southern Sudan.\(^{92}\) Krik suggested the importance of PKDL as human VL reservoir.\(^{94}\)

1.2.1.1.5 Transmission:

Undoubtedly, Sandfly transmission by P.orientalis is the major route of infection in Kala-azar. The occurrence of Kala-azar cases in military patrols in uninhabited areas suggest azoonosis.\(^{77}\) Rapid transmission in families or village units was noted Van Peenen and Reid who found clustering of cases in one household, Possibly caused by aman-Sandfly-man cycle or by man-to-man transmission. Crowding, Poor nutritional condition and dermal involvement (ulcers) were factors to be considered. Eltoum et al, described the first case of congenital transmission of Kala-azar in the Sudan, this rare mode of transmission has been reported sporadically from other endemic areas.\(^{77}\)
1.2.1.2 Clinical Presentation

The Incubation Period of VL in human is typically 2-6 months but may be shorter or much longer, the onset may be gradual or a cute.\textsuperscript{5,43,80}

Most reports indicated a strong male preponderance, in particular the hospital-based studies. Most studies report cases to be more frequent in late childhood and early adult life, although in field studies especially in outbreaks all age groups were affected and cases were seen in children less than 1 year of age.\textsuperscript{77}

Fever and splenomegaly were present in the vast majority of patients. Moreover, abdominal pain, weakness and epistaxis were the most common symptoms recorded in several studies. In one large study weight loss was recorded and found in all 99 studied cases. Hepatomegaly and lymphadenopathy were also commonly found. Asymptomatic and prolonged subclinical forms of VL infection are common in endemic areas. The ratio of subclinical to clinical was found to be 1:1.4 - 1.6 in Sudan.\textsuperscript{77} However in Kenya and Italy the ratio was 5:1.\textsuperscript{3}

The first documented case of VL, followed by dermal and mucocutaneous leishmaniasis, was described by Kirk and MacDonald. It was found that Kala-azar could be accompanied by skin lesions invariable numbers of cases up to 50%; lesions were described as crusted ulcers, Papules, Verrucose nodules or small ulcers. Involvement of the mucous membrane of the mouth may occur starting with gingivitis and subsequent loosening of teeth, as reported by Sati.\textsuperscript{77}
1.2.1.3 Diagnosis

1.2.1.3.1 Parasitological methods:

Demonstration of leishmania parasite by direct microscopy or by invitro culture of aspirates from the spleen, bone marrow remain thegoldstandard for the diagnosis of VL.\textsuperscript{4}

**Spleen aspiration**

Is the most sensitive method; splenic smear has a sensitivity of 95-97\%.\textsuperscript{25,95} The prothrombin time should not be more than 5 sec. Longer than the normal control value and the platelet count should not be below 40000/mm\textsuperscript{3} in patient in whom spleen aspiration is to be carried out.\textsuperscript{3}

**Bone marrow aspiration**

The superior posterior iliac crest is most often selected, but others prefer the sternum. It can be done in hospital as well as in the field, but it is not suitable for mass screening. In Sudan a sensitivity of 70\% and 80\% has been reported for bone marrow smear.\textsuperscript{25,85}

**Lymph node aspiration:**

It is mostly safe, easier, with less pain, and more acceptable by the patients. In Sudan, sensitivities of 52\%, 59.3\% and 80\% were described for lymph gland smear.\textsuperscript{25,96,97}
Other body materials:
Parasite may be found in all organs in the body, including the blood. In Sudan Rhor had confirmed the occurrence of the parasites in the peripheral blood of 35% of VL patients in Kapoeta district.\textsuperscript{31}

Cultures and animal inoculation:
Material obtained by lymph node, bone marrow or spleen aspiration may be used for culture. NNN, Schneider’s and RPMI are the most commonly used media.\textsuperscript{4} The sensitivity of in vitro culture method in detection of L. donovani parasites in Sudan was estimated to be 9%.\textsuperscript{85} however ElDawi described a sensitivity of 92% in a similar investigation.\textsuperscript{98} A sensitivity 100% for in vitro culture using NNN medium was reported.\textsuperscript{43}

Syrian hamsters are the most suitable animal and may be inoculated by the intraperitoneal or intracardic routes. Rhors reported a sensitivity of 45% for animal inoculation.\textsuperscript{31}

1.2.1.3.2 Serological methods
Their main asset is non-invasiveness, but they all have restrictions in common. Differentiation between clinical and subclinical or past infection is not possible in some of them. Titers may remain high for years and cannot be used for evaluation of therapy. Cross reactions with other pathogens, e.g. trypanosomes may occur depending on geographical location. Some serological techniques need expensive, sophisticated equipment which precludes their use in remote areas.\textsuperscript{20}
Direct agglutination test (DAT):

The cut-off titer is a round 1:3200, but should be established for every endemic area. Titers 1:3200 were found in all 40 parasitologically confirmed patient and in 9 other clinically suspected patients who responded to therapy in a study in Hawata rural hospital (Eastern province)\(^5\)\(^9\). EL- Safi and Evans, found similar results in the hospital for tropical Diseases in Omdurman; 25 confirmed and 9 clinical suspected patients had DAT titers 2 1:12800. Healthy British and Sudanese control subjects had titers < 1:400.\(^{100}\)

A study in Gedaref State, Sudan, revealed a sensitivity of 96 and specificity of 99% with a cut-off titre of 1:8000.\(^{101}\)

Enzyme-linked immunosorbent assay (ELISA):

In Sudan the standard ELISA was evaluated, and compared with the DAT. All sera of parasitologically confirmed VL patients were positive in both tests. In patients with cutaneous leishmaniasis, sensitivity of the DAT was 67% and that of the ELISA was 60%.\(^{97}\)

Indirect immunofluorescence test (IFAT):

Abdalla recorded a sensitivity of 100% and specificity of 97.7% in the diagnosis of VL in the Sudan. Normal controls (300) gave positive results in 3.3% of individuals, but only at the lowest dilution (1:100).\(^{102}\)

Counter-immunoelectrophoresis (CIE):

It has been reported that the test has a sensitivity of 72% and specificity of 98.7% in diagnosis of VL.\(^{102}\)
**Latex agglutination test (LAT) for serum:**

A sensitivity of 88 % and specificity of 100 % have been reported. LAT is a simple and practical serological technique for the diagnosis of VL, particularly at dispensary level.\(^{103}\)

**Non-specific serological diagnostic tests:**

These include the albumin / globulin ratio and the formol gel test (FGT), both based on the high immunoglobulin levels found in VL.

These testes have poor sensitivity and specificity.

**1.2.1.3.2 Tests of Cellular immunity:**

**Leishmanin skin test (LST):**

LST or Montenegro test is a delayed-type hypersensitivity (DTH) reaction. It is positive in 61% of Sudanese PKDL patients and negative in active VL. A positive LST indicates that a treated VL patient has develop cell-mediated immunity to leishmania.\(^{50}\)

**1.2.1.3.4. Nucleic acid hybridization**

Two studies in Sudan on cases with confirmed VL showed sensitivity of 70 % and 92.5 % respectively.\(^{104,105}\)

**1.2.1.3.5 Latex Agglutination test (Katex):**

Recently a latex agglutination test for the detection of urinary antigen has been developed\(^{(6)106}\) which is simple, rapid, economical and most suited for use in remote rural areas. The test had 100 % specificity and a sensitivity of 81.4 % in human urine samples which is comparable to bone marrow aspiration.\(^{96}\)
experimentally infected animals' urine antigen was detected as early as one week post-infection, and more importantly, the antigen level started to decline very quickly after treatment. In the light of the encouraging results obtained recently in the hospital setting in the Sudan, a trial of the test at the furthest possible peripheral levels of the health care system will be highly justified. Such test is particularly needed in the remote areas where the disease is endemic and where poor accessibility makes it difficult to use the normal clinic based diagnostic methods.
1.2.2 HIV in Sudan

HIV/AIDS is an emerging health problem in the Sudan which is located in a very unstable region and bordering countries with high HIV prevalence. Socioeconomic factors and large population movements due to the civil war in the southern region may play an important role in the spread of HIV. The cumulative numbers of AIDS cases reported by the end of 1997 were 1873 cases. This number has risen to 2067 by the end of June 1998 and to 8222 cases by January 2002. About 69% of AIDS cases were males with a prevalence of 91% among adults between 15-50 years. The major mode of transmission is Heterosexual route. However these figures may not reflect the exact situation, as there are many unclear reporting. The estimated number of HIV sero-positive individuals in the country is 180,000 by 1997 with a prevalence of 99% among adults. It has been observed that there is an increasing trend in HIV sero-positivity among both low and high-risk groups. The regions mostly affected are the southern and eastern regions. Last surveillance results of 1997 in Gedarif state showed prevalence rate of 5% among antenatal clinic attendants (0% in 1991), and 14% among T.B patients 7% in 1992; in southern Sudan a prevalence of 5% and 17.4% for antenatal T.B patients respectively, was found.
1.2.3 Leishmania-HIV co-infection

1.2.3.1 Epidemiology of leishmania-HIV co-infection:

A total of 1663 co-infection cases from 25 countries have been reported to the WHO’S Division of Control of Tropical Diseases. Leishmania-HIV co-infection has been regarded as an emerging infectious disease, especially in Southern European countries, where up to 70% of all adult cases of VL are related to HIV/AIDS and, up to 9% of all AIDS cases suffer from newly acquired or reactivated VL.\textsuperscript{10} By contrast, very few cases have been reported from countries outside the southern European countries, particularly from sub-Saharan Africa.

There is no doubt that the number of documented cases substantially underestimates the actual number of cases because of problems in recognition, diagnosis and reporting of either HIV infection, or leishmaniasis, or both, in the setting of developing countries. This is supported by the fact that co-infection has been documented in only a few countries from sub-Saharan Africa.\textsuperscript{112} although the existence of the problem has been reported to the WHO by more than ten countries. Moreover the WHO thinks that, in southern Europe, the available numbers are still underestimated.

There have been major epidemiological changes in Europe in recent years that give cause for further concern. In southern Europe, visceral leishmaniasis used to be traditionally among children, whereas today 73\% of co-infected patients are injecting drug users. Similarly, in the Mediterranean area, where visceral leishmaniasis was traditionally zoonotic (the dog being the only source of infection for the sandfly), cases have recently arisen where transmission has been from person to person, either through
the vector, or through syringes. Co-infected patients can serve as human reservoirs, harbouring numerous leishmania in their blood and becoming a source of infection for the vector. Co-infected patients, who are often injecting A drug users, can also transmit the leishmania among themselves through needle sharing. If the number of co-infected patients continues to increase, the risk of epidemics in the Mediterranean basin is likely to increase accordingly.\textsuperscript{10}

Figure (1): (cases reported to WHO 1998)\textsuperscript{10}
1.2.3.2 Gaps in the Reporting System Worldwide:

While becoming progressively more realistic, the number of cases reported in the world is still considered an underestimation. A lack of awareness, rare systematic detection, limited access to HIV tests, absence of notification, non-compulsory notification of leishmaniasis, and limited number and coverage of the surveillance centers, all contribute to an under-reporting of HIV-related cases. In 1998, in Brazil, India, Kenya, Nepal and Sudan, where there is co-infection, the numbers reported are disproportionately low. In Nepal, for instance, it is estimated that 25% of the Nepalese sex workers become HIV positive after three years of activity in a neighbouring country: Their return to their native rural areas—highly endemic for visceral leishmaniasis creates the conditions for co-infection. But, surveillance systems have only just been set up with financial support. Similarly, the surveillance centers in India have just recently been financed and staffed, because the leishmaniasis/HIV overlap is increased in the Bihar and West Bengal States. Ethiopia is a good example, however, of a country where the detection, management and reporting of co-infection cases is already well organized. The number of cases reported in the two and a half years between 1996-1998 was three times that of the number of cases reported between 1990-1995. On the other hand, there is no leishmaniasis surveillance system in West Africa, as this disease is not a public health problem there.

In Europe, a surveillance system is now well-established creating greater awareness, improved detection of both diseases and better case reporting. Overall case detection, however, remains passive.
Closing the gabs in active medical surveillance requires financial support, staff, and facilities for diagnosis, as well as an extensive communication network.\textsuperscript{10}

1.2.3.3 Clinical Presentation of Leishmaniasis in HIV-Positive Subjects:

1.2.3.3.1 Introduction:

HIV modifies the clinical presentation of leishmaniasis in the co-infected patient. The majority of clinical cases of leishmaniasis in HIV-positive individuals in Spain display clinical features of classic kala-azar.\textsuperscript{8,13} However, cases of CL and MCL, as well as VL in many atypical locations, have been increasing in the past years. This has led physicians to suspect that almost every organ containing phagocytic cells may eventually become infected by \textit{L. donovani}. Furthermore, several atypical etiologic agents have been described in leishmanial syndrome affecting HIV-infected subjects.\textsuperscript{4} Four major characteristics can be established for HIV-associated leishmaniasis, all related to the immunologic impairment caused by the virus: (1) parasitic dissemination, to the skin in DCL, or throughout the reticuloendothelial system in visceral and visceralizing syndrome; (2) atypical locations; (3) a chronic and relapsing course\textsuperscript{(11)113}, with each patient typically experiencing two or three relapses despite proper treatment\textsuperscript{8}; and (4) poor response to standard therapy.\textsuperscript{114} Lack of anti-leishmania antibodies is a fifth characteristic seen in Spain and many endemic areas. However, Gari-Touissant et al have described a rate of serologic positive findings that reached 100% in patients infected with HIV in the South of France.\textsuperscript{115}
1.2.3.3.2 Age and Sex distribution:

There have been major epidemiological changes in VL pattern in Europe in recent years. In southwestern Europe, VL used to be traditionally a childhood disease, whereas today the co-infection mainly strikes adults, 76.9% of co-infected patients are aged between 31 and 50 years. The age distribution of co-infected patient is consistent with that of IVDUS compared to our previous analysis (WHO/LEISH/ 96.39), the main age has risen slightly. Most of the patients (83.2%) are males, which is consistent for all age groups.

1.2.3.3.3 Population at risk:

In southwestern Europe, there is a clear predominance (71.1%) among intravenous drug users (IVDU), who are clearly identified as the main population at risk. Globally, the proportion of homosexuals is almost the same (11.1%) as that of heterosexuals (11.4%), but at country level there are significant differences. In contrast with IVDU, these two risk groups are less important in co-infection cases than in HIV/AIDS patients.116

Figure 4:
1.3.3.4 Clinical Forms

VL (kaIa-azar) in HIV-Positive Patients:

The most frequent clinical spectrum occurring in co-infected persons is VL, although various cutaneous forms of the disease have also been described. The incubation period is variable and may be age-related. Although a median incubation period of three months is accepted for VL in immuno-competent individuals, fulminant presentation of VL is possible in patients with AIDS. Moreover not all infections are symptomatic, relapses are usual, and HIV-infected patients who are IVDUS (46% in Spain) may be at high risk of acquiring Ichishmania parasites via needle-sharing.

It is estimated that 2 to 9 percent of patients with AIDS acquire VL in southwest Europe. VL is the fourth most common opportunistic disease in HIV-positive individuals in Spain after pneumocytosis, toxoplasmosis, and cryptosporidiosis. Most patients (80-85%) are male, and have fever, hepatomegaly and/ or splenomegally, hypergammaglobulinemia, and pancytopenia.

A review of current literature indicated that 20 to 40% of the cases had absence of splenomegaly; however it has been reported that only 12% of cases lacked splenomegaly. Anaemia is multifactorial, and related to antiretroviral therapy, autoimmune haemolysis, splenomegaly, and gastrointestinal blood loss. Gastrointestinal blood loss is exacerbated by thrombocytopenia. Agranulocytosis, cancrum oris, and super infections complicate untreated cases. Constitutional symptoms (asthenia, anorexia, loss of weight) are seen in approximately 70% of co-infected patients, and lymphadenopathy in 60%. Other manifestations such as hyperpigmentation or tongue changes are rarely seen.
As a matter of fact, the disease tends to disseminate to the skin and other organs, and presentation outside the reticuloendothelial system may mislead the clinician.\textsuperscript{7} VL is considered to be similar to disseminated tuberculosis, because both conditions are produced through latent infection reactivation.\textsuperscript{2}

Gastrointestinal symptoms are among the most frequent complains in individuals with HIV infection who experience these symptoms during the course of the infection. Immunological impairment allows disseminated infection or localized disease of the gastrointestinal tract, with a wide array of opportunistic pathogens, including protozoa. Cryptosporidium species, Isospora B. belli, microsporidian protozoa, Giardia lamblia, and Entamoeba histolytica are the most commonly identified causative agents of these protozoa infections. \textit{L} donovani has been identified in the digestive tracts of 50% of HIV-positive patients with VL, and it is increasingly being reported in HIV-positive patients. Any portion of the gastrointestinal tract can undergo digestive parasitization; jejunal involvement has most frequently been found on biopsies, but esophageal, gastric, duodenal, ileal, colonic, and rectal involvement have also been reported.\textsuperscript{2}

However, the site of the digestive tract that is most frequently involved in gastrointestinal leishmaniasis is unknown.\textsuperscript{114} The symptoms depend on the predominant digestive focus involved. In HIV-negative patients, the major digestive symptoms of VL include diarrhea, malabsorption, hypoalbuminemia, and weight loss. In HIV-positive patients, diarrhea is the most common finding, but vomiting, dysphagia and/or odynophagia, abdominal pain, epigastric pain, gastrointestinal bleeding, and rectal discomfort can also be expected. Profuse diarrhea may appear as a
prominent symptom, eventually leading to malabsorption, hypoalbuminemia, or even prerenal insufficiency.

Unfortunately, these symptoms are completely unspecific in the patient with AIDS, soon extensive differential diagnosis must be ruled out. In addition, leishmania parasites may coexist with many other pathogens or neopalsms in a single digestive area, thus making it very difficult to ascertain the contribution of each infection to the gastrointestinal symptoms. Leishmania amastigotes can be identified on hematoxylin and eosin staining of gastrointestinal mucosal biopsy specimens.2

Cutaneous involvement associated with VL is a rare finding, even in HIV-positive patients, but it is especially characteristic of HIV-related VL. Cutaneous involvement is seen in 2 to 12% of cases of HIV-related VL. L. donovani has been enveloped in dermal lesions associated with VL. Such lesions may occur simultaneously with visceral involvement and can be maculopapular or nodular. In addition, mucocutaneous and mucosal leishmaniasis in kala-azar has been described, especially in Sudan.38 Romeu et al first described a case of VL involving the lung and a cutaneous Kaposi’s sarcoma lesion in an HIV-infected patient. Since then, several similar reports have appeared. Leishmanial amastigotes are commonly found in Kaposi’s sarcoma cutaneous lesions concomitant with VL. The association between VL and Kaposi’s sarcoma is probably explained by the good perfusion and large amount of small vessels in Kaposi’s sarcoma lesions that can potentially act as a filter for the parasites. Leishmania parasites were recently found in herpes zoster lesions on an HIV-positive patient. Leishmaniasis has also been reported presenting as a dermatomyositis-“like
eruption in three patients with AIDS. Dauden et al have suggested that leishmaniasis may be included in the list of diseases capable of inducing a dermatomyositis-like eruption.²

Atypical clinical findings in HIV-related VL suggest that immune failure may facilitate parasittemia and haematogenous spread of leishmaniasis from typical locations to every part of the body. In fact, amastigotes of leishmania species can be observed in the peripheral blood of HIV-positive patients². Moreover, using xenodiagnosis viable parasites were found to circulate in peripheral blood, and where recovered from all 10 patients tested². Indeed haemoculture has proven useful in 67% of 25 tested patients with average CD4 +ve cells counts of 45/mm³ (all bellow 150/m³) however the authors suggest that haemoculture would probably be less efficient for patients with high CD4 +ve counts. This haematogenous dissemination of VL in HIV-positive patients would explain the atypical harvests reported to date. Importantly, atypical locations can be the first clinical manifestation of VL in the immunodepressed patient. Pancreatic, pulmonary, and pleural leishmanial infections have been described. Gonzalez-Anglado et al² reported AtWo cases of laryngeal leishmaniasis in HIV-infected patients who presented with a six and nine month history of hoarseness. No clinical or histopathological evidence of VL was seen in the first patient until nine months later, when clinical symptoms of VL appeared and bone marrow biopsy finally disclosed a multitude of leishmania amastigotes and a culture yielded promastigotes. A necropsy study in an HIV-VL co-infected patient revealed numerous parasite forms located in both adrenal glands, and three focal points were discovered in the left cardiac ventricle and in other more common locations. Adrenal
insufficiency and myocarditis should probably be expected in HIV-related VL, but further confirmatory reports are needed.  

1.2.3.4 Immunopathology of leishmania-HIV-Coinfection:

Both leishmania and IHV are able to infect and multiply in monocyte/macrophage cells. Interestingly, following a primary infection both pathogens can establish latent infection. Infection by two different intracellular pathogens of the same cell target will have important effects on the immune response and might influence the expression and multiplication of either one, or both.  

1. 2.3.4.1 Effects of HIV that Favor Leishmania Multiplication:

Under normal circumstances, T-lymphocytes respond to leishmania antigens by proliferation and production of lymphokines. These lymphokines, mainly interferon gamma (INF-γ), and probably other yet unknown factors, can then activate oxygen-dependent and –independent microbicidal systems in the macrophages which are essential defense mechanisms against intracellular amastigotes. Some patients may be unable to restore lymphokine production and microbicidal mechanisms even after treatment with aritimonials, thus permitting amastigotes to persist in macrophages eventually for life. When they face new immunologic depressor factors, infection may be reactivated. Clinical cases have been seen several years after exposure. When faced with immunologic depression, the balance would be off, allowing an accelerated multiplication of the amastigotes and the invasion of the viscera. Reactivation of latent VL has been recorded in association with chronic diseases in patients treated with steroids and other immunosuppressor agents and in various
subsets of V immunocompromized hosts, such as patients with hematological neoplastic and autoimmune disease, or renal transplant recipients.\textsuperscript{2}

Nonetheless, it should not be forgotten that the wide majority of cases of leishmania infection reactivation seen in the Mediterranean basin are associated with HIV-related immunosuppression. Although the proportion of monocytes infected with HIV is believed to be very low.\textsuperscript{112}

a number of monocyte/ macrophage dysfunctions have been documented during HIV infection. These include impairment of chemotaxis, accessory cell function, major histocompatibility complex class II (MHC-II) expression and, intracellular killing activity\textsuperscript{112} as well as reduced production of INF -y.\textsuperscript{2} These dysfunctions have been ascribed to the effects of cross regulatory and inhibitory Th-2 cytokines, to defects in the inductive signals that activate macrophages and to the direct immunosuppressive effects of HIV or its envelope glycoproteins.\textsuperscript{112}

including decreased CD4 +ve cell counts.\textsuperscript{2} Therefore, it is not surprising that HIV can modulate the intracellular growth of leishmania in macrophages, facilitate the spread of leishmania protozoa, and diffuse visceral parasitization, resulting in unusual locations of these organisms, 2 poor response to standard therapy, and relapse.\textsuperscript{113}

1.2.3.4.2 Effects of illeishniania that Favor HIV Replication:

It has been proposed that chronic immune activation by parasitic infections is an important factor in the pathogenesis of HIV disease progression. They suggested that this might be particularly relevant
in the setting of the developing countries, especially sub-Saharan Africa, where the rate of HIV infection is accelerated. Tropical parasitic and other helminthic infections are highly prevalent in these countries.\(^1\)

These are believed to serve as co-factors in the pathogenesis of the accelerated rate of HIV disease progression.

Leishmania derived antigens might directly activate HIV-1 replication in a nonspecific manner. This notion is supported by the recent findings that direct stimulation of latently infected CD4+ve T-cell lines by (Lipophosphoglycan) can result in virus expression without the need for antigen presentation by macrophages. It has been demonstrated that leishmania, and its major membrane antigen LPG, can induce the activation of HIV in latently infected monocytoid cells. This mechanism of HIV-1 activation involving the cytokine TNF-\(\alpha\) is non-antigen specific. TNF-\(\alpha\) induced by leishmania-infected macrophages may also result in the expression of HIV in CD4 +ve T-cells in a paracrine manner. IL-6, "INF-\(\alpha\) and granulocyte-macrophage colony-stimulating factor (GM-CSF) have been shown to play a major role in up regulating HIV gene expression by either a transcriptional or a posttranscriptional mechanisms.\(^2\) L donovani has a role in cytokine transactivation of latent HIV-infected cells by their ability to secrete such cytokines.\(^2\) Peripheral blood B cells from patients with active VL are polyclonally activated.\(^1\) Thus, these activated B cells may be capable of inducing HIV expression in vivo by virtue of their ability to secrete cytokines, especially TNF-\(\alpha\) and IL-6. Another possibility is that "enhancing" antibodies may facilitate infection of macrophages, which normally express low levels of CD4, by opsonization of the virus-antibody complex.
Another proposed imbalance that might contribute to disease progression involves the specific Th effector population. While subsets of CD4+ T-cells designated as Thl (which produce the cytokines IL-2 and IFN-γ) protect against pathogens that are removed primarily through cell-mediated immunity, Th1 subsets (which produce the cytokines IL-4, IL-5, IL-6 and IL-10) protect against pathogens removed primarily through humoral mechanisms. Moreover, Th1 and Th2 cells cross regulate each other and, thus, cytokines produced by one Th subset can suppress the production and/or activity of the others subsets. Both leishmania and HIV alter the Thl-Th2 balance. Leishmania tends to depress the activity of Th1 and/or induce the activity of Th2 cells.

It has been proposed that a switch from a Thl to a Th1 cytokine profile is associated with progression of IHV infection.\textsuperscript{112}

It has been shown that cytotoxic T-cell activity is depressed in patients with active VL. Thus, dysfunction of these cell subsets might result in increased susceptibility to HIV infection and/or disease progression in HIV-infected persons, as CD8+ T-cells can suppress HIV replication in vitro.\textsuperscript{112}

\textbf{1.2.3.5 Lab diagnosis of co-infection:}

The diagnosis of VL in leishmania/ HIV co-infected patients is particularly difficult. The usual clinical features of VL such as fever, weight loss, and swelling of the liver, spleen and lymph nodes, are not always present or may be hidden by other associated opportunistic infections with similar symptoms.\textsuperscript{116}
1.2.3.5.1 Serological Diagnosis:

There is a negative humoral response in 42.6% of co-infected patients. However, there are major differences between the tests and the laboratories.

Gari-Touissant et al\textsuperscript{115} described a leishmania seropositivity rate that reached 100% in patients infected with HIV in the south of France. However, in most of the endemic areas of the Mediterranean basin, most HIV-seropositive patients with VL display an absence of anti-leishmania antibodies. However, it is recommended by the WHO that, to use 2 or more serological tests and antigens freshly prepared in the laboratory to increase sensitivity.”\textsuperscript{6} Methods used in antibody detection include enzyme-linked immunosorbent assay (ELISA), direct agglutination test (DAT) and immunofluorescence assay test (IFAT). Use of Western Blot (WB) has been recommended to release the number of false-negative results produced with these techniques.\textsuperscript{117}

1.2.3.5.2 Parsitological Diagnosis:

The diagnostic tools used for each leishmanial syndrome, cutaneous, visceral, and mucocutaneous disease vary somewhat, but the gold standard in each case remains the isolation or identification of the parasite from appropriate tissue. Evans et al\textsuperscript{7} provided an accurate description of the procedures. No good evaluations of the sensitivity of the different diagnostic tools for HIV-positive patients are available. For VL in the immunocompetent patients, best samples are those obtained from spleen aspirations (sensitivity> 94%), liver biopsies (sensitivity =76% to 90%), and bone marrow biopsies (sensitivity= 76% to 90%).
1.2.3.5.3 Polymerase Chain Reaction (PCR):

Ravel et al\textsuperscript{2} described a highly sensitive, simple, and rapid PCR procedure which allowed direct detection of leishmania in PBMCs of the patient with no target DNA purification. Other PCR-based diagnostic procedures\textsuperscript{117} have also been shown to be more rapid, sensitive and specific and less disturbing for patients than conventional diagnostic procedures. Although the cost of PCR-based diagnostic methods has been reduced by using “inexpensive” reagents, it still precludes its use in underdeveloped countries.
<table>
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<tr>
<th>Test</th>
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<th>Positive</th>
<th>pos.%</th>
<th>Negative</th>
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<td>753</td>
<td>93.54</td>
<td>52</td>
<td>6.46</td>
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<td>211</td>
<td>145</td>
<td>68.72</td>
<td>66</td>
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<td>S Skin</td>
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### 1.2.3.6 Treatment of HIV-Related Leishmaniasis:

Primary drug failure and subsequent clinical and parasitologic relapse following initial drug therapy characterize the response of HIV co-infected patients to treatment. Persistence after human infection leadsto recrudescent disease if cell-mediated immunity is compromised.\(^2\)

Lifelong maintenance therapy is indicated in HIV co-infected patients, although optimal agents and regimens remain to be determined.\(^6\)

### 1.2.3.6.1 Antimonials: (SbV):

SbV, first used in the 19405, is still the mainstay of therapy.\(^6\) Recent studies and treatment regimens have emphasized the need to use higher doses than those recommended in the past, in
addition, a previous total dose limit of 850mg/day was recently replaced with a recommendation of no upper limit.²

1.2.3.6.2 Amphotericin B (AMB):
Is the most active antileishmanial agent in use.¹¹⁸ Its toxic effects, however, have limited its use in humans largely to cases of SbV-resistant mucosal leishmaniasis. Liposomal AMB (L-AMB) has been effective in the primary treatment of VL in both immunocompetent and immunocompromised patients, including those infected with HIV. In addition, striking clinical and parasitological responses to L-AMB, with none of the toxic effects normally associated with the use of standard preparations of AME have been reported in several HIV-positive Patients.²

1.2.3.6.3 Therapeutic Combinations:

1.2.3.6.3.1 Allopurinol:
Laguna et al.¹¹⁹ showed that allopurinol Plus inegluimine antimoniate induced clinical improvement in 82% of 11 HIV-VL co-infected patients. A synergistic effect was noted with the combination of allopurinol and stibogluconate.¹²⁰

1.2.3.6.3.2 INF-γ:
INF-γ enhances the ability of macrophages to eliminate intracellular pathogens; Exogenously administered IFN-γ augments the capacity of macrophages to eliminate leishmania infection in vitro models and acts synergistically with pentavalent antimonials.² Different groups have demonstrated the safety and efficacy of parenteral recombinant human IFN-γ in combination with pentavalent antimonials in patients without AIDS.³
1.2.3.6.4 Treatment of Relapse:

In HIV-positive patients, immune system impairment contributes to relapses. Up to 50% of patients experience a relapse six months after treatment and 90% after one year. These recurrences may be treated again with SbV. However, its efficacy may diminish, perhaps due to the development of resistance to antimonials. Treatment with L-AMB has been demonstrated to be highly effective without any associated toxic effects. An alternative approach to relapses includes retreatment with AMB lipid complex (Abelcet).

1.2.2.4 Leishmania/ HIV Co-infection in Sudan

In View of the fact that both VL and HIV infections are increasing in the Sudan particularly in eastern and southern states where both infections overlap, VL cases co-infected with HIV may be found. The reported cases in Africa are a modest estimation. Surveillance was set up in Kenya and Sudan, only in 1998.

In Sudan 1990-1998 only 3 cases of Leishmania and HIV Co-infection were reported. The numbers are expected to rise owing to factors such as increasing mass migration, displacement, civil unrest, war and sex work.

El-Safi et al, have confirmed the prevalence of Leishmania/HIV Co-infection in Sudan. 6.5% of VL cases have been found to be co-infected with HIV. Most (75%) HIV Co-infected cases come from eastern and southern Sudan. This is in accordance with the fact that these are the two most affected areas in the country with regards to both VL and HIV infection.
Generally HIV co-infected VL cases presented with clinical features typical of VL although splenomegally was found only in 75% of the cases.\textsuperscript{107}
2. Materials & Methods

2.1- Study design:
A descriptive cross-sectional, hospital and Community-based study.

2.2- Study area:
A- HIV laboratory (National health Laboratory. Khartoum).
Samples form i- Khartoum State hospitals.
   ii- Jouba town hospitals.

B- Kassab and Um El-Kheir Centers (Under the responsibility of Medicines sans frontieres (MSF) Holland. Gedarif State, Eastern Sudan.

2.3- Samples:
1. 125 confirmed HIV Positive serum samples.
2. 185 serum samples from VL suspects.
3. 185 urine samples from the same VL suspects
4. 128 Gland puncture smear from VL suspects.

2.4- Research team:
1. The Supervisor.
2. The Author
3. Lab. Technicians.

5. Driver

**2.5- Data analysis:**

SPSS- based analysis.

**2.6- Ethical consideration**

A written consent was taken from the Humanitarian Aid commission (Khartoum). As well as the Ministry of health Gedarif State.

**2.7- Material and Equipment**

- Microscope (Olympus CH\textsubscript{2})
- Refrigerator
- Freezer (-70 c)
- Cooled incubator
- Balance
- Spectrophotometer
- Power supply (CD)
- Ice box
- Elisa reader c
- PH meter
- Vertical gel electrophoreses apparatus Mini-protean 3 cell (Bio RAD)
- Transfer tank (Bio - RAD)
- Rotator
- Tray with channel
- Microtitre plate flat bottom 96 well (Nunc immuno plate Brand products)
- Microtitre plate V shape
- Tips different size
- Automatic pipettes different capacity
- Cryogenic tubes
- Vacationer (sst gel and clot activator)
- Tissue culture flask different size
- Flacon tubes (25 and 50 ml size)
- Teflon coated slide
- Whatman filter paper 3 mm
- Gloves
- Forceps
- Humid chamber
- Tissue paper
- Spectrophotometer
- Nitrocellulose paper
- Scissors
- pipettes
- tourniquets
- 21 G needle
- 5 mL syringe
- Diamond pencil
- Methanol
- Slides rack
- Stock Siemsa stains
- Buffere, PH 72 solution
- Sterile blood lancets
• Hard card
• Clips
• Gelatin
• Yellow tips
• Combitip 2.5 ml
• Normal saline
• DAT antigen aqueous
• Foetal calf serum
• V shape microtitre plate

2.8- Reagents:

Preparation of solution

phosphate buffered Saline (PBS-PH. 7.2)

-Sodium chloride = 8 gram
-Potassium chloride = 0.2 gr
-KHZ po4 = 0.2 gr
-N32 Hpo4 12 HzO = 2.88 gr
-Distilled water = 1 liter

Dewqycholit acid sodium salt monohydrate (Doc)
1.0 gram in 10 milliliter Distill - Water

Alkaline phosphate bufler (Apb - ph 9.5) 10 x can

- Tris base sigma 60.5 gram
- Sodium chloride 29.0 gram
- Distill - Water 500 ml
- Magnesium chloride 5.0 g

First dissolve tris-base and sodium chloride in 400 ml Distill-Water adjust pH to 9.4 using HCL, then add MGCL. Complete with Distill Water to 500 milliliter.

- **Skimmed milks**

It is dried free fatty milk used in a concentrations 0.5% to dilute the tested sera as well as secondary antibodies (anti-lgG) also it a best blocking agent, it block the peripheral internal surface of the micro plate wells to smooth the reaction.

- **10 % Ammonium per sulfate**

0.277 mg + 2.77 ml Distill-Water (1 gram in 10 ml)

This concentration must be prepared freshly a few minutes before use or it may stored in 40°. The solution provides free radicals that drive polymerization of acrylamide and biacrylamide

- **10 % Sodium dodecyl sulfate (SDS)**

10 gram of sodium dodecyl sulfate (Sigma L-450 g) is dissolved in 100 ml Distill-Water and stored at room temperature. It help pattern migration of polypeptides.

5 M of Sodium chloride (Nacl)

Dissolve 292.2 gram Nacl in one liter of Distill-Water stored at room temperature.

- **Tris base 2- Amino- 2 hydroxy meth y L-1-3 propandiol (sigma T-1503)**

  a. Tris base [MPH 8.0
Dissolve 121 gram tris- base in 600 ml Distill-Water

- **Adjust PH to 8.0 using Hcl then Complete the volume to 1000 ml**

b. Tris bas 1.5 MPH 8.8

- Dissolve 90.5 gram 300 m1 Distill-Water
- Adjust PH to 8.8 using Hcl, then complete to 500 ml

c. Tris- base 0.5 M pH 6.8

- Dissolve 30 gram of tris base in a few amount of Distill-water.
- Adjust PH then complete with Distill-Water to 500 ml.
- All the tris solution must be stared in refrigerator

- **EDTA 0.5MPH 8:**

Dissolve 46.5 gram EDTA powder in 250 ml Distill-Water

Adjust PH using Na on

- **Preparation of Resolving gel 12 % for tris - glycine SDS - polyacry lamid gel electrophoresis.**

  - Distill-Water 1.6 ml
  - 30 % acrylamide mix (Bio Red) 2.0 ml
  - 1.5 M Tris PH 8.8 1.3 ml
  - 10 % SDS (Sigma L-3771) 0.05 ml
  - 10 % ammonium Persulfate 0.05 ml
  - TEMED (sigma T-8133) 0.002 ml

(N, N, N, N, tetramethyle thylenediamine)
• Preparation of 5 % stacking gel
  ▪ Distill-Water 0.68 ml
  ▪ 30 % acrylamide mix (Bio Red) 0.17 ml
  ▪ MtrisPH6.8 0.13 ml
  ▪ 10 % SDS (sigma L-3771) 0.1 ml
  ▪ 10 % ammonium per sulfate 0.1 ml
  ▪ TEMED (sigma T-8133) 0.001 ml

(N, N, N, N, tetramethyle thylene diamine) it is accelerates the polymerization of acrylamide and biacrylamide by catalyzing the formation of free radical from ammonium per sulfate

• Tris Bujeure Saline TBS
  ▪ tris 1.0 M 25 ml
  ▪ Nacl 5.0 M 30 ml
  ▪ D.H20 945 ml 7
  ▪ Triton x 100 solution (sigma) 100 n]  

• Sample loading Buffer

Tris 0.5 M PH 6.8 825 pl
EDIA 0.5 M 60 ul
Sucrose (sigma S-7963) 2.15 gr
Bromophenol blue 0.1 % 60 ul
SDS 10 % 2 ml
D.H20 7.9 ml

• Tris - glycine electrophorus ’s buffer

a 5 x stock solution is made by dissolving
Tris - base (sigma T-1503) 45.3 gram
glycine (sigma G- 4392 ) 282 grm
SDS (sigma L 3771) 15 grm
D.H₂O 3000 ml

working solution is prepared by mixing 60 ml of stock solution with 240 ml D.H2O this is sufficient for one tank.

- **Transfer buffer (Blotting buffer) PH 8.3**
  
glycine (sigma G - 4392 ) 14.5 g
  Tris base (Sigma T-1503 ) 29 g
  Methanol 1000 ml
  or ethyle alcohol absolute C₂H₆O
  complete with DHzO to 5 liter

- **Preparation of coating bufler carbonate \ bicarbonate buffer (0.1 m) PH 9.2**
  
  A. Solution (A)
  
  Sodium carbonate (sigma S 6139) 10.6 g
  D.H₂O 1000 ml
  
  b. Solution (B)
  
  Sodium bicarbonate (sigma S 6014) 8.4 g
  Dis- H20 1000 ml
Mix solution A and B by adding A to B adjust PH to 9.2. Or by adding 40 ml of solution A to 460 ml of solution B this give a PH 9.2

- **Giemsa Stain : Stock solution**
  - Giemsa powder 3.8 g
  - Glycerol (glycerin) 250 ml
  - Methanol (Methry alcohol) 250 ml

The contain was mixed well and heated at 50-60 °C up to 2 hour. Then the stain was stoppered in bottle and stored at room temperature.

- **Buffered saline PH. 7.0 -7.1:**
  - disodium hydrogen phosphate 10 g
  - Dis – H₂O water 1000 ml

From the mixture 0.4 - 0.7 ml was mixed with 500 ml physiological saline. This is working buffer

2.9 Diagnostic Techniques:

2.9.1 Gland Puncture

The slide was cleaned with gauze and laboelled with patient name or lab number. The Patient was rested on his /her back with the legs stretched out. The skin over the inguinal gland was disinfected with 70 alcohol and then allowed to dry.

The gland was held between thumb and index finger of the left hand. The (21 G) sterile needle was inserted in the center of the
gland at right angles to the skin, the gland was squeeze with the left hand while the needle rotated. The fluid then Come up throughout needle, the needle was rapidly drawn and the swab with disinfectant was applied over the site of puncture.

The piston of the syring was drawn back and the needle containing L.N aspirate was attached. The fluid was discharged on the slide and quickly spreaded as thin smear. The slide smear was air dried, and then dipped in 100 % methanol for one minute for fixation.

The fixed smear slide was placed in a trough or in staining rack and 1:10 concentration of Giemsa stain was purred over the slide and left for 10 minutes. The slide was then rinsed briefly with tap water, and then dried on slides rack at vertical position. The smear was examined under microscope at 100 x with drop of oil. The a mastigotes was identified as small round or oval body with pale blue cytoplasm and red large nucleus and kinetoplast photo (1 1) inside or outside the macrophage cells.

2.9.2 Serological Methods of VL:

2. 9. 2. 1 Enzyme - linked Immunosorben Assay (ELISA)

- Test procedure
  a- coating plate

1- Carbonate \ bicarbonate buffer (0.1 M. pH 7.2 ) was used to dilutethe Antigen. The required concentration was 10 ug/ml of antigen per well.

2- 100 microliter of the diluted antigen were dispensed to each well of a flat bottom microtiter plate (NUNC- Immuno maxisorp)
3- The plates were incubated over night at 4-8 °C

b- Washing:

On the next day the plates were taken and the fluid were discarded by hand flicking. Immediately all the wells were filled with washing buffer (TBS+ Triton ) using bottle squeeze . Washing procedure was carried out three times, then the plate was inverted and daped on tissue paper several time (3 x) to remove the rest fluid

c- Testing Sera:

Each test serum was diluted 1/100 in 0.5 % skimmed milk (casino) with tris phosphate buffer (TBS) and 100 111 of the diluted sample was added into, each well . Positive and negative control sera were included in each plate. Well A and B in column one were used for blank and only substrate were added in them . After incubation at room temperature for 60 minutes on rotator, the plate was washed as mentioned earlier . Then100 ul of alkaline - Phosphatase-conjugated . goat anti-Human IgG(H+L) ( Jackson immuno research) diluted 1/10,000 in 0.5 % skimmed milk were added into each well except blank . Then the plate was incubated for 60 minutes at room temperature on rotator and then washed . 100 111 of substrate (PNPP ) alkaline phosphatase substrate,(sigma fast N- 2770). The substrate must be prepared in distill water, afew minutes before adding. Then the plate was incubated in the dark for 90 - 120 minutes at room temperature

Reading were carried at 405 nm , on spectrophotometer. An OD value of 0.5 or more was considerd as positive ( 2 0.5 ) and less than ( < 0.5) was regarded as negative photo (12).
2.9. 2.2 Indirect fluorescent (IFAI)

Test procedure:

1- The serum samples were heated in water bath at 56 c° for 30 minutes.

2- Each serum sample was diluted 1:50 with phosphate buffered saline (PBS).

3- The substrate slides were taken out from -20 and allowed to reach room temperature. Then fixed in acetone for 10 minutes.

4- Substrate slides were labeled according to the testes sample number, and placed in the humid chamber.

5- 50 microliter of diluted 1:50 serum were added to appropriate circle. Positive and negative control sera were included.

6- After 30 minutes of inculcation time at 37 0° the substrate slides were removed from humid chamber and thoroughly rinsed with PBS, and then placed in rack and immersed in PBS and agitated on rotator for 10 minutes.

7- The boundary of the slides was dried with tissue paper and returned to the humid chamber.
8- Immediately each circle was covered with 50 ul of fluorescent conjugate (anti-human IgG developed in Rabbit sigma F-4512) 1/100 dilution in PBS.

NB : The wells must not be left uncovered for longer than 15 second

9- The conjugate was allowed to react for 30 minute at 37 co , in the dark , then washed as in step (6)

10- The slides were removed one slide at a time from washing rack and solution around the wells was quickly dried

11- One drop of mounting medium (glycerin) was added to each well , and covered with cover slip avoiding air bubbles

12- . A drop of glycerin was placed on the cover slip and the slides were viewed under fluorescence Microscope 40 x objective after dropping of glycerin.

**Interpretations:**

The test was considered positive if they fluorescence bright apple green and negative if they were dull red in colour photo (13).

**2.9. 2.3 Western blotting**

Sodium dodecy sulfate (SDS) Polyacry Iamide gel electrophoresis:

All analytical electrophoresis of protein were carried out in acrylamide gel . The protein dissociated into their individual polypeptide subunit. SDS was used in combination with a reducing agent and heated to dissociate the proteins before they are loaded on the gel.
1- A clean dry glass plate was assembled as shown in photo (14) gel cassette 

Appropriate volume of 12 % resolving gel was rapidly purred into the gap between two plates.

2- A sufficient space , about the length of the teeth of the comb plus one centimeter was left for stacking gel.

3- Immediately the acrylamide solution was over layed with 200 pl of butanol.

4- The gel was allowed to polymerize for 1-2 hour at room temperature.

5- The butanol then rinsed off with distill water, and the remaining water was removed with a paper towel or filter paper.

6- Stacking gel solution was directly poured onto the surface of the polymeried resolving gel completely filling the gap, aclean Teflon comb was immediately inserted into the gel cassette, avoiding air bubbles , and more stacking gel was added filling the comb space completely.

7- The gel was allowed to polymerize for 1-1 1/2 hour at room temperature.

8- Teflon comb was removed gently from the gel , and the gel was rinsed thoroughly with Dis.H₂O.

9- Then the gel cassette was mounted on electrode assembly . Tirs glycine electrophoresis buffer (nmning buffer) was added 125 ml to the inner chamber , and 200 ml to the outer tank.

\textbf{a- Sample loading}
The antigen sample was mixed with loading buffer so as to have 200 ug/ml a final concentration, plus 10 ul of 2-Mercapto-ethanol (2ME) per gel.

Also rainbow marker (Arnersham pharmacia biotch) was prepared by mixing equal amount of rainbow marker with loading buffer. The mixtures were heated at 100 °C for 3-5 minutes. 200 pl of the prepared sample was loaded with Hamilton syringe or pipette tip on the center of the gel, also 10 pl of rainbow mixture was loaded at the margin of the gel near the spacer in a groove made for it.

b- Gel electrophoresis

The tank of electrophoresis was covered, and then connected to the power supply, the apparatus was supplied with 100 - 120 volts for 1 ½ - 2 hour, then the power supply was turned off and electric lead disconnected, the inner chamber assembly was taken, the fluid (rurming buffer) was discarded.

c- Transferring

Nitrocellulose’s sheet (PVDF gelman) 10 cm x 7 cm was prepared. Gloves and forceps were used as the paper must not be touched with fingers.

The prepared membrane was floated with distilled Water for 5 minutes. Then immersed in transfer buffer.

The transferred apparatus was arranged as follow, Rack, porous pad on it, then two layer of filter paper (whatman 3 mm) wetted in
b- Substrate

Alkaline phosphatase substrate solution was prepared a few minutes before adding to the membrane. The lyophilized vial which contain 50 mg Nitro blue tetrazolum NBT (sigma N-6876) reconstituted with 660 pl of 70 % N.N. Dimethyl forrnamid DMT (sigma D-8654 And the vial that contain 50 mg of 5- Brame - 4 - chloro - 3- indolylphosphate BCIP (sigma B-8503 ) was reconstituted with 100 microliter of Pure DMF, then the vials were stored at -20 0° until use The working solution was prepared out from above two reagents , by diluting 33 pl of NET and 25 pl Bcip in 20,000 pl of alkaline phosphate buffer.

1500 microlilter of mixed reagent were added into each channel. The plate was agitated to allow the substrate to react with the probed stripe, the reaction was stopped after observation of a suitable color intensity then thestripes were dried on air. To avoid the fading of color the stripes were sealed under plastic and stored in the dark photo (16).

2.9. 2.4 Direct agglutination test (DA T)

Collection of sample:

The whatman No 3 was cut into small pieces, the patient name and date of sampling was written on it at one end A blood sample was collected by finger prick, two punches on filter paper.

The blood must be saaked through the filter paper. The sample was clipped on hard card and dried on air.

Elation of sample:
The working sheet and microtitration plate was prepared and marred with sample number. 5 mm of blood sample was punched out from filter paper and placed in the wells of the first column of microtitre plate. Each sample is plated in different well according to the registration of working sheet.

125 μl of normal saline was added into each well containing sample.

The plate was covered and incubated over night (at least 8 hours), at 4 °C (fridge).

**Preparation of diluent and titration**

The antigen, foetal calf serum (FCS) and DAT plate were taken out from fridge 50 ml of normal saline were mixed with 0.5 ml FCS, and 390 μl of 2ME was added to the mixture and mixed together. Also a mixture of 50 ml of normal saline with 0.1 gram gelatin were heated in water bath for 10 minute plus 390 μl of 2ME.

The dilution of serum in column one was 1:50. 50 μl of diluents was dispensed into each well starting from column 2 up to column 12. The content of column one was mixed 5X and 50 μl of it was taken to column 2 and then mixed and 50 μl of it were transferred to the next column and so on up to column II. From column II 50 μl was discarded, and 50 μl diluent was placed in column 12 (negative control).

The antigen bottle was rotated gently to resuspend the sedimented promastigotes. 50 μl of antigen was added into each well, starting from column 12 to column 2. The plate was covered and rotated clockwise and anticlock wise up to 10 times. The plate was
incubated on a quite leveled surface for 12-18 hours at room temperature

**Reading DAT plate**

The plate was placed on White back ground and the titre was estimated compared with negative control. The titre was the last dilution that showed difference compared to negative control photo (17).

**Interpretation**

For simplification the titre was written as the column No of the well

<table>
<thead>
<tr>
<th>Column</th>
<th>Titre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colum 2</td>
<td>1:100</td>
</tr>
<tr>
<td>Colum 3</td>
<td>11200</td>
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<tr>
<td>Colum 4</td>
<td>1:400</td>
</tr>
<tr>
<td>Colum 5</td>
<td>1:800</td>
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<tr>
<td>Colum 6</td>
<td>1: 1600</td>
</tr>
<tr>
<td>Colum 7</td>
<td>1: 3200</td>
</tr>
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<td>Colum 8</td>
<td>1:6400</td>
</tr>
<tr>
<td>Colum 9</td>
<td>1: 12800</td>
</tr>
<tr>
<td>Colum 10</td>
<td>1: 25600</td>
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<tr>
<td>Colum 11</td>
<td>1: 51200</td>
</tr>
<tr>
<td>Colum 12</td>
<td>control</td>
</tr>
</tbody>
</table>

**Sera:**
In the case of sera obtained from venous blood samples, 2 pl of each serum sample was diluted with 100 pl of diluents in column 1 to give 1/50 dilution. The titration and other stapes were followed as in the case of sample eluted from finger prick blood spots on filter papers.

2.9.3 Latex agglutination test Kautex for urine antigen detection:

**Kit components.**

- 5 ml antibody coating test latex in dropper bottle
- 5 ml positive control in dropper bottle
- 5 ml Negative control in dropper bottle
- Black glass slide with four reactions marked zones
- Wooden mixing stick
- Empty sample tubes
- Sample pre-treatment

Sufficient samples tubes were labeled for each patient to be tested 250 pl to 1000 ul of each urine sample were transferred into sample tubes

All the urine sample in tubes were put in a rack, immersed in boiling water and heated for 5 minutes, then the urine was allowed to cool to the ambient temperature before applying the test.

**Latex test:**

All reagents were brought to room temperature and the bottle of latex was shaken well immediately before testing. 50 ul of the treated urine sample were placed in the reaction zone on the glass slide, then one drop of well mixed latex was added.
Both were mixed until the liquid was completely homogeneous covering the whole surface of the reaction zone.

The glass slide was tilted by hand, clock wise and anti clock wise continuously for two minutes, then the degree of agglutination was recorded under a good stream of daylight photo (18).

**Interpretation of results**

Agglutination was interpreted as follows:

+++ Majority of the latex agglutinates and moves to the edge of the reaction zone.

++ Clear agglutinated particle were seen against a background

+ Agglutination was just visible compared to the negative control

- No visible agglutination

**2.9.4 serological test for HIV:**

- Enzyme-linked immunosorbent assay (ELISA).

for HIV screening and confirmation.

**- Step 1:**

1- Microtiter strips were washed immediately before use by the working wash solution (3a) 400 ug/ well, Incubated for 30 sec and
aspirated off carefully. The remaining liquid removed by tapping the plate upside down on tissue paper.

2- 100 pl of dilution buffer (2) were pipetted into all wells.

3- The following quantities were added

i- 20 ul negative control (4) to wells BI/CI/DI.

ii- 20 ul positive control (5) to wells EI/F 1.

iii- 20 ul patient sample into remaining wells.

4- Incubated for 30 min at 37 °C

1- The conjugate working solution (6a) was papered

2- The contents of the wells were aspirated into 5 % sodium hypochlorite solution, and 400 ul working wash solution were added to each well and then aspirated after about 30 sec and this wash well was repeated 4 times

- Step II:

1. 100 ul of conjugate working solution (6a) were Dispensed into all wells.

2. The Plate was incubated for 15 min at 37 °C

3. Substrate working solution (7a), was prepared

4. The strips were washed 5 times as described in step I no. 6

-Step III:

1- 100 pl of substrate working solution (7a) were dispensed into all 9 wells.
2- The plate was incubated for 15 min at room temperature

3- 100 pl of stop solution (9) were dispensed into all wells, and the absorbance was measured within 30 min against the blank Al at 450nm using a reference wavelength between 620 and 690 nm.

**Calculation:**

The mean absorbance of the negative control (MN C) and positive control (MPC) were calculated at 450 nm.

\[
MNC = \frac{A_{450}(B1) + A_{450}(C1) + A_{450}(D1)}{3}
\]

\[
MPC = \frac{A_{450}(E1) + A_{450}(F1)}{2}
\]

The cut-off value (COV) was calculated as follow:

\[
COV = MNC + 0.300
\]

**Interpretation of Results:**

- Samples with absorbance values \(A_{450} \leq COV\) were considered reactive for Anti-HIV antibodies.
- Samples with absorbance values \(A_{450} < 0.9 \times COV\) were considered negative for anti-HIV antibodies.
- Samples with absorbance values \(A_{450}\) between 0.9x COV and COV are equivocal.
- Reactive samples were confirmed by another 2 different ELISA of the same procedures detecting different anti-HIV antibodies.
Chapter Three

Results
3. Results

3.1 HIV + ve individuals screened for VL:

Table (1) and Table (2) Show the serological results of HIV +ve individuals in Khartoum State (95) and Juba town (30) respectively who have been screened for VL by 4 serodiagnostic tests: DAT, ELISA, IFAT and WB.

The DAT was positive in 4 (3 %), the ELISA in 37 (30 %), the IFAT in 10 (8 %) and the WB in 6 (4.8 %) of the 125 studied samples (Fig. 1 - 4).

Table (3) shows that 45 (36 %) of the studied samples showed evidence of specific antileishmania antibodies. None of samples was positive by 4 methods. 34 (27 %) were positive by one method, 8 (6%) by two methods and only 3 (2.5 %) by the three methods.
If only the samples that were positive by two or more serological tests are considered positive, then the prevalence of leishmania infection in the HIV +ve individuals is stag/a \(\frac{11}{125}\) as shown in Fig (5).

Table (4) and Fig 6 - Fig 8 show the demographic and serological findings of HIV positive individuals co-infected with leishmania as indicated by at least two serological tests for “Quick males and only one was a female. Their age ranged between 4 and 50 years (mean \(27\)). 8 of them were AIDS cases and 2 were asymptomatic individuals who had been identified during the routine screening of travelers and blood donors for HIV. The positivity of the different serodiagnostic tests in these individuals was as follows in a descending order: ELISA 100\% \(\frac{11}{11}\), IFAT 54 \% \(\frac{6}{11}\), WB 45 \% \(\frac{5}{11}\) and DAT 27 \% \(\frac{3}{11}\).

3.2 VL cases screened for HIV:  

Between July 2001 and December 2001. A total number of (185) VL suspects were screened for HIV. (129) at Kassab and (56) at Um El-Kheir centres.

Table (5) and table (6) show the demographic, parasitological and serological results of the studied cases. 137 were males (74 \%) and 48 were females (26 \%). 61/133 (45.8 \%) were children 1-15 years.

The GP was positive in 37 (29 \%) of the patients, the DAT was positive in 96 (81 \%), the Katex in 127 (69 \%), the ELISA in 158 (89 the IFAT in 156 (88 \%), and the WB in 153 (86 \%) of the 185 studied samples Fig. (9 - 14).
Screening for HIV showed 8 (4\%\%) reactive samples and the confirmatory tests indicated that (4\%) were confirm cases.

Table (7) showed the demographic, parasitological and serological finding of the VL cases co-infected with HIV.

All the confirmed leishmania /HIV co-infected cases were males, the Known ages of two of them varied between 30 -40 years. One was parasitologicaly confirm by positive GP. The all confirm 4 cases were strongly positive by ELISA, IF AT, WB and Katex. They were all from Kassab center.

Table 1: Serological results of HIV positive individuals in Khartoum state screened for VL

<table>
<thead>
<tr>
<th>Serial No</th>
<th>Code No</th>
<th>DAT</th>
<th>ELISA</th>
<th>IFAT</th>
<th>WB</th>
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<td>-</td>
<td>-</td>
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</tr>
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<td>+</td>
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</tr>
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<td>+</td>
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<tr>
<td>64</td>
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Table 2: Results of HIV positive samples from Juba town screened for VL
Table 3: HIV positive samples that showed specific antileishmania antibodies by different serological tests: DAT, ELISA, IFAR and WB.

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Table 4: Demographic and serological finding of HIV positive individuals co-infected with Leishmania

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Table 5: Demographic, parasitological and serological findings of VL cases at Kassab centre

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Table 7: demographic, parasitological and serological findings of HIV positive cases at Kassab and Um El-khair centre
• Confirmed.
• NK: Not Known
Figure (6): The serological results of HIV positive individuals
Screened for VL by the DAT
Figure (7) : The serological results of HIV positive individuals screened for VL by ELISA
Figure (8) : The serological results of HIV positive individuals screened for VL by the IFAT
Figure (9) : the serological results of HIV positive individuals screened for VL by WB
Figure (10): Evidence of specific antileishmania antibodies by different serological tests (DAT, ELISA, IFAT, and WB) in HIV + ve samples.
Figure (11) : SEX distribution of HIV / leis mania co – infected individuals, (n = 10)
Figure (12) : age distribution of HIV / leis mania co – infected Individuals , (n =9)
Figure (13): The state of HIV / leis mania co – infected individuals
Figure (14) : katex results of at kassab and Um Elkeir Centers
Figure (15): ELISA results of VL suspects at kassab and Um Elkheir centers
Figure (16): IFAT results of VL suspects at kassab and Um EL – Kheir
Figure (17): WB Results of VL suspects in kassab and Um EL – Kheir
Figure (18) : seroprevalence confirmed of HIV + ve individuals in VL cases (kassab and Um El – K heir center )
Figure (19): DAT results of VL suspects at Kassab and Um EL-kheir centers

- Positive: 81.4%
- Negative: 18.6%
Figure (20): GP results of VL suspects at kassab and Um EL – kheir centers
Chapter Four

Discussion, Conclusion & Recommendations
4.1 Discussion

4.1.1 HIV individuals Screened for VL:

The incidence of leishmaniasis as an opportunistic infection in AIDS patients increased substantially since the first case of HIV-leishmaniasis co-infection was described in 1985124. It has been reviewed that both leishmania and HIV can infect and multiply in macrophages, and both of them can dysregulate the immune response. On one hand leishmania can induce the activation of HIV in latently infected monocytic and T cells, and on the other hand HIV can enhance and modulate intracellular growth of leishmania in macrophages. Thus a full blown picture of both VL and AIDS are expected to increase in areas where the two infections overlap. The large population movement toward big towns from the peripheral rural areas endemic for VL, and civil war in the Sudan in addition to socioeconomic factor may contribute to the overlap of the two infections. The major areas of the overlap between L. donovani and HIV infection in the Sudan are located in the Eastern and Southern states, where co-infection can be an emerging problem that need special attention.

The results of the present study indicate that 36 % of HIV positive individuals who have been diagnosed at the National Health Laboratory; Department of virology in Khartoum, had evidence of specific antileishmania antibodies. As only those who are positive by two serological methods are considered positive for VL according to WHO criteria.116 only 8.8 % of the studied individuals were also positive for VL. This finding confirms the prevalence of subclinical leishmania infection in those individuals. As Khartoum and Juba states; from where the samples were collected;
have not been considered as VL endemic areas it can be assumed that those individuals were originally coming from or had a history of travel to VL endemic area. Such in formations were not available to us due to the retrospective design of the present study. It would have been of great interest if those co-infected individuals could have been followed up for a considerable period of time to study the evolution of both infections.

In addition, parasitological studies on those with the full blown picture of AIDS might have confirmed VL, the diagnosis of which can be difficult due to atypical clinical presentation. Similar findings have been reported from the Mediterranean area. In Spain, it has been reported that 2-9% of AIDS cases acquire VL.²

The present study showed that the different serological methods vary in their sensitivity for the detection of specific antileishmania antibodies in HIV positive individuals. The highest sensitivity 30% was obtained with ELISA, followed by the IFAT (8%) while the DAT was the least sensitive method. Surprisingly the WB, which has been considered as an extremely sensitive test was positive in only 4.8% of the studied individuals. It was interesting to note that a child of 4 years was among the AIDS/leishmania co-infected individuals in our studied series. Although the general consensus that HIV transmission is heterosexual in the Sudan, the present study has demonstrated the possibility of congenital transmission which constitute a considerable problem in other African countries such as Tanzania, Kenya, Zimbabwe and South Africa.
4.1.2 VL Suspects Screened for HIV:

The seroprevalence of HIV infection among patients with VL in endemic area (Kassab and Un El-Kheir centers) was 2% in the studied cases. This is a bit higher than the prevalence of HIV in general population in Sudan (1.6%), and lower than the one that was earlier obtained by (EL-Safi et al) (6.5%). Comparatively the low prevalence that we obtained in our results was likely to be due to low prevalence of HIV in Sudan. In Ethiopia it was found that the HIV infection is 7-10 times more frequent in VL patients than in the general community.

Among a series of 140 VL confirmed patients in north-western Ethiopia which is bordering the endemic area in eastern Sudan, 28 (20%) were HIV sero-positive. In this area there is an intense migration of refugees, returnees, seasonal workers and commercial sex workers. In the Mediterranean basin up to 70% of adult cases of VL are associated with HIV infection.
4.2 Conclusions

The following conclusions can be drawn from the present study:

1- 8.8% of HIV positive individuals are co-infected with leishmania infection.

2- The most sensitive method for the detection of specific antileishmania antibodies in HIV positive individuals is the ELISA, followed by the IFAT and the WB. The least sensitive is the DAT.

3- Congenital transmission of HIV amounts to in HIV/leishmania co-infected individuals in this present study.

4- The prevalence of HIV among VL cases (8.1%) Most of the cases show evidence of humoral immune responses to leishmania.
4.3 Recommendations

1- Further studies are required to determine the magnitude of HIV/leishmania co-infection which is a recognized emerging problem in Sudan.

2- Prospective studies are needed to investigate the impact of HIV on leishmania infection and vice versa.

3- Anti-retroviral therapy should be considered for the treatment of proven individuals co-infected with leishmania, together with specific treatment for VL.

4- HIV infection should always be excluded in case of VL individual not responding to anti leishmania therapy.

5- Record system concerning the HIV/leishmania co-infection should be stablished.

6- HIV/VL co-infected individual should be faced and followed up.
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