Liver and Renal Profiles in Kala-azar, HIV and Kala-azar patients co-infected with HIV in Gedarif and Sinnar States.

Sudan.

By:

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A Thesis Submitted for Fulfillment of Master Degree in Medical Laboratory Sciences (Chemical pathology)

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This thesis is dedicated to Nada, the sun that sends its shines generously to my sky, the music that revives my silent universe and the rose that pinks my grey life...

Your lucky husband
Ayman
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Last but not least, I would like to thank my family for their continuous support and encouragement.
**Abbreviations**

AIDS: Acquired Immune Deficiency Syndrom
ALP: Alkaline Phosphatase
ALT: Alanine transferase
AST: Aspartate transferase
APCs: Antigen Presenting Cells
ART: Anti Retroviral Treatment
BMA: Bone Marrow Aspiration
CD: Cluster Differentiation
CDC: Center for Disease Control
CFT: Complement Fixation Test
CMV: Cytomegalovirus
CPK: Creatine phosphokinase
CSF: Cerebro Spinal Fluid
CTL: Cytotoxic Lymphocyte
DAT: Direct Agglutination Test
DF: Dilution Factor
DNA: Deoxy Nucleic Acid
EDTA: Ethylene Diamino Tetra-acetic Acid
ELISA: Enzyme Linked Immuno Sorbent Assay
EMRO: Eastern Mediterranean Region
ESR: Erythrocytes Sedimentation Rate
FACS: Fluorescence Activated Cells Sorter
GAG: Glucose oxidase Anti-Glucose oxidase
Hb: Hemoglobin
HIV: Human Immunodeficiency Virus
IDPs: Internally Displaced Peoples
IFAT: Immuno Florescence Antibody Test
IHC: Immuno Histo Chemistry
INF: Interferone
KATEX: Kalazar Agglutination Latex
LAT: Latex Agglutination Test
LDb: Leishman Donofani bodeis
LDH: Lactate dehydrogenase
MAC: Mycobacterium Avium Complex
MCL: Muco Cutaneous Leishmaniasis
ME: Mercapto Ethanol
MHC: Major Histo Compatibility
mRNA: Messenger Ribo Nucleic Acid
MW: Molecular Weight
NASBA: Nucleic Acid Sequence Based Amplification
PBP: Peripheral Blood Picture
PCR: Polymerase Chain Reaction
PCV: Packed Cell Volume
PKDL: Post Kalazar Dermal Leishmaniasis
PMNC: Peripheral Mono Nuclear Cells
RBCS: Red Blood Cells
RIA: Radio Immuno Assay
RNA: Ribo Nucleic Acid
RT-PCR: Reverse Transcriptase Polymerase Chain Reaction
RTU: Ready To Use
SNAP: Sudan National AIDS Program
STD: Standard
TB: Tuberculosis
TBS: Tris Buffer Saline
TCR: T. Cell Receptors
TGF: Tumor Growth Factor
TNF: Tumor Necrosis Factor
tRNA: Transfere Ribo Nucleic Acid
TWBCS: Total White Blood Cells
VL: Visceral Leishmaniasis
WB: Western Blott
WHO: World Health Organization
Abstract

**Background:** Visceral Leishmaniasis (VL) presents a major health problem in several states in Sudan. VL/HIV co-infection is an emerging health problem which has been reported to be associated with renal and liver dysfunction.

**Design:** A descriptive study and cross sectional studies also were performed.

**Setting:** The study was conducted at Tabarak-Allah Rural Hospital Gedarif state and Al- Azaza Centre, Sinnar State.

**Objectives:**
- General objective: To study the prevalence of VL/HIV co-infection in the Sudan
- Specific objective: To determine the liver and renal profiles in VL, HIV and VL/HIV co-infected patients.

**Material and methods:** A total of 99 VL parasitologically confirmed cases in Tabarak-Allah Rural Hospital (Gedarif state, Eastern Sudan) and Al- Azaza Centre (Sinnar State) and. There were 57(57.6%) males and 42(42.4%) females, their ages ranged from 2-75 years (mean of ages was 16 years), 30 HIV seropositive individuals from Al Gadarif Teaching Hospital were studied, 23 (76.7%) of them were males, and 7 (23.3%) were females. Their age ranged between 30 and 54 years (mean of age was 40 years). All serum samples from VL samples were analyzed for renal and liver profiles using chemistry analyzer and were also screened for HIV by third generation ELISA kits. The 30 HIV seropositive samples were analyzed for liver and renal profiles and for CD4 cell count by flowcytometry.

**Results:** Of the 99 VL confirmed cases jaundice was detected in Nine (9.1%), elevated AST activity in 85(85.9%), elevated ALT in 19(19.2%),
elevated ALP in 55(556%), low albumin level in 78(78.8%), hyperproteinaemia in 40 (40.4%), azotamia in 7 (7.3%), hypocalcaemia in 48 (48.5%), hyponatraemia in 89(899%), hypokalama in 29(29.3%) and hyperkalaemia in 11(11.1%). Two (2.4%) patients had renal failure.

Seven (7.1%) of the 99 confirmed VL patients were HIV co-infected. One (14.3%) of those 7 HIV/ VL co-infected cases had elevated ALT, 7(100%) had elevated AST, 6(85.7%) had elevated ALP activity, 2(28.6%) had hyperproteinaemia, 5(71.4%) had hypoalbuminaemia, 3(42.9%) had hypocalcaemia, 7(100%) had hyponatraemia, 2(28.6%) had hypokalama and 1(14.3%) had hyperkalaemia.

Of the 30 HIV seropositive individuals, 3(10%) were jaundiced, 12(40%) had elevated ALT, 11(36.7%) had elevated AST, 11(36.7%) had elevated ALP, 6(20%) had hyperproteinaemia, 3(10%) had hyperproteinaemia, 19(63.3%) had hypoalbuminaemia, 3(10%) had Azotamia, 16(60%) had hyponatraemia, 2(6.7%) had hyperkalaemia, 10(33.3%) had hypokalama, 13(43.3%) had hyperuricaemia, 1(3.3%) had hypouricaemia, 16(53.3%) had hypocalcaemia and one (3.3%) had renal failure.

The comparison of liver profile between confirmed VL and VL/co-infected cases showed significant differences in ALT, ALP, AST, T.Protien and Albumin Levels (P.Value < 0.05) but there was no significant difference in T.Bilirubin and D.Bilirubin levels (P.Value > 0.05). In the renal profile creatinine and sodium levels, were also significantly different between the two groups (P.Value < 0.05). However, there was no significant difference in urea, calcium and potassium levels (P.Value > 0.05).
Conclusion and Recommendation: In view of the results of the present study it is concluded that VL and VL/HIV co-infection can be associated with impairment in renal and liver functions; . The prevalence of VL /HIV co-infection is increasing in Sudan. Accordingly, it can be recommended that renal and liver profiles should be determined before treatment in all VL cases that show clinical features of renal or liver dysfunction. Urine analysis is recommended to be a routine test in VL as an indicator for any abnormality in renal function. Due to the limited number of VL/HIV co-infected cases in the present study further studies involving larger sample size are required.
المستخلص

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وإصابة

: شدة مدى الرصد وآكثر، الحشوة الشعرية الشمانية، تدأب المريض على الكلي، واضحة هو تدأب في الكبد، انزياحات النشاط في الملاحظة والإرتفاع الإيقان، أظهرت الكبد، وظائف والعلاج المرضي وإصابة المشتركة الإصابة المريض وآضاء،

 السودان في ملاحظة تزايد في النعامة النقص الفيروسي، انتشار معلق، خطورة صحية متشكلة يشكل واصبح.

 أين يظهر الذين الأسلاك مع التحقيق، هو الحشوة الشمانية بدء الصحة حالات في وخصوصية بهذه يوحي أن يمكنك الكلى وظيفة في خاصة ويتكلس

 الكبد وظائف ضعيف علامة الذين يظهر العلاج أثناء،

 إذا أظهر hỏi الذي يظهر الرنين هذه التحليل، إيحاء الكلي وظيفة في يحتوي ووجد علية تدل سريري الأعراض الذي يظهر العلاج في
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I. Introduction & Literature review

1.1. Leishmaniasis Overview:

Leishmaniasis is an infection caused by protozoan of the genus *Leishmania* of Trypanosomatidae family. The infection is transmitted by infected female sand flies of the genus *Phlebotomous* in the Old World and *Lutzomyia* in the New World. About 30 species of *Leishmania* are known to infect man leading to a wide clinical spectrum. These include three forms of leishmaniasis; namely the cutaneous (CL), the mucocutaneous (MCL) and the visceral leishmaniasis (VL) (WHO, 1990).

About 35 million were believed to be at risk of having leishmaniasis in about 88 countries where as 12 million having been reported to be currently infected (Salotra et al., 2001). Without prompt appropriate treatment, as many as 95% of kala-azar patients die, resulting in at least 50,000 deaths per year worldwide (Kolaczinski et al., 2008). However the actual number of cases estimated to be three to five times higher than the number reported as leishmaniasis usually occurs in remote areas where other health problems are predominant (WHO, 1993).

The World Health Organization (WHO, 1990) recognized the disease as one of the most important parasitic diseases in the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases (TDR). VL affects at least 12 million people worldwide with an annual incidence of 500,000 new cases (WHO, 1996). Previous epidemics of VL involving hundreds of thousands of people had occurred in India, Bangladesh and Sudan (WHO, 1996). The disease is fatal, with a mortality rate reaching approximately 100% among untreated cases (WHO, 1996). But some 95% recover if treated in time (IRIN, 2009).
Of the 500,000 new cases of VL which are estimated to occur annually; 90% come from five countries Bangladesh, Brazil, India, Nepal and Sudan. VL is caused by *L. donovani* in the Indian subcontinent and in east Africa, by *L. infantum* in the Mediterranean region and by *L. Chagasi*, which is closely related to *L. infantum*, in the new world mainly in Brazil, Peru and Paraguay (WHO, 1993).

The disease results from multiplication of the parasites (*Leishmania* amastigotes) within the mononuclear phagocytes of the reticulo-endothelial system. The classical clinical features of VL are fever, weight loss, pancytopenia and on physical examination, hepatosplenomegaly with the spleen generally more enlarged than the liver, typically 5-15 cm below the left costal margin (WHO, 1996). Splenomegaly may be absent in 5% of cases (Nandy, 1998). Malnutrition is an established risk for development of VL in childhood (Yousif, 1967). Histologically, the parasites may be found in the macrophage of the spleen, liver, lymph node and even the skin. Immunologically VL patients show a selective energy to *Leishmania* antigens, first characterized by a negative Leishmanin test, which may fail to convert even after cure (Elhassan *et al.*, 2001).

**1.2. Historical background of VL:**

Kala-azar first came to the attention of Western doctors in 1824 in Jessore, India (now Bangladesh), where it was initially thought to be a form of malaria. Assam gave kala-azar one of its common names, Assam fever (Mosby’s Medical Dictionary, 2009).

Visceral and cutaneous leishmaniasis were known as far back as the first century A.D in central Asia. VL has been known in the old world in India since the 19th century by the name black fever or Kala-azar due to skin darkening. A mastigotes were first seen in the skin of a patient with "Delhi boil" in Calacuta by Cunningham in 1885 (WHO, 1984). William Leishman in 1903 was able to identify the organism in the splenic smear of an Indian patient who died "with
Dum Dum" fever, another name for VL. At the same year Donovan recognized the same intracellular parasite, seen by Leishman, in the spleens of three cases in Madras, who presented with fatal disease that was considered as chronic malaria. Hence the organism was named Leishman Donovan body in honour of these two pioneers in the field of leishmaniasis. Rogers in 1904 succeeded in culturing the parasite responsible for VL from splenic aspirates (Dedet et al., 1999).

1.3. Epidemiology of VL:

Leishmaniasis is a worldwide disease which is found in all continents, except Australia (Manson & Bahr, 1987). However it is most common in the tropics and sub-tropics. Certain factors contribute to determine its distribution such as high humidity and its mean diurnal variation (Napeir, 1946). There is a definite seasonal variation, most commonly in Autumn following rainy seasons, as in Bengal and, Endemic areas of kala-azar exist in the Sudan and these are confined to districts with a minimal annual rainfall of 10 inches; the disease does not occur in the northern arid areas of the Sudan. (Robert, Hasseeb. 1937) or in early summer as in China or in Spring as in Europe. VL is found in Asia, Southern Europe, particularly in Greece, Southern France, Spain. In South America it is found in Brazil and Venezuela. In Africa, the disease is found in Sudan, Ethiopia, Somalia, northern Kenya, Equatorial West Africa, Morocco, Algeria and Tunisia, (Manson & Bahr, 1987).

1.4. Parasites of VL:

Leishmaniasis is caused by protozoan parasites of the genus Leishmania which belongs to the family Trypansomatidae. It has different species which are responsible for the three clinico-pathological conditions; (a) Visceral leishmaniasis which is caused by L. donovani complex (b) cutaneous-leishmaniasis caused by L. tropic, L. major, and L. mexicana and (c) muco cutaneous Leishmaniasis which is caused by L. braziliensis and L. mexicana complexes (WHO, 1990). The L.
The Leishmania parasites assume two forms, the flagellated form (infective stage) named promastigote and the non-flagellated form (amastigote). The promastigote form is 15-30 μm long found in the gut of the sand fly (the vector) and can be cultivated in culture media. It has a single flagellum, arising from a blepharoplast at the anterior end and a nucleus. A mastigote is the intracellular form which is oval or round measuring about 2-3, μm in length (Manosn & Bahr, 1987). It is found in the mammalian host and can be recovered from all endothelial cells, including reticulo endothelial system, bone marrow, lymph nodes, spleen and liver, as well as from monocytes, polymorphs and the submucosa of the respiratory and alimentary tracts (Napier, 1946). It can also be recovered from stool or urine (WHO, 1997).

1.5. Life Cycle:

L. donovani is an obligatory intracellular parasite which cycles between the mid gut of sand flies (extracellular promastigotes) and the phagolysosome of mammalian macrophages (intracellular amastigotes) (Dedet et al., 1999). Following inoculation of promastigotes into the skin of mammalian host by a sand fly (Phelebotomns orientalis) while seeking a blood meal at night, they soon lose their flagella, transform into the amastigote form and become engulfed by macrophages. Inside the macrophage they multiply by binary fission. The macrophage eventually ruptures when heavily parasitized to release parasites which are engulfed again by other new macrophages. The new amastigotes penetrate the cell membrane and are ingested by the sandfly, when it seeks its next
blood meal at night (Cheesbrough, 1995). Then each amastigote regains its flagellum, after going through many development stages. The sandfly becomes infectious within 10-15 days of the blood meal (Manson & Bahar, 1987).

1.6. Reservoir hosts:

There are two types of reservoir hosts: wild reservoirs such as canines which include foxes, wolves and jackal and domestic animals like dogs, rodents and rats (WHO, 1990).

1.7. Vectors and transmission:

VL is transmitted from one mammalian host to another including man by the female sand fly which has different species, geographical and seasonal distribution. Sandflies are mainly in the tropics and sub-tropics. In the following countries, the most common species include: in Sudan Phlebotomus papatasi (7%) and P. orientalis (5%) were sympatric, mainly inside homes (85% and 75%, respectively (Dereure, El-safi, et al.2003), P. martins in Kenya, P. major and P. pernicious in the Mediterranean, P. chinensis in China and P. argentipes in India.

Other modes of transmission do exist although their occurrence is rare. These include blood transfusion, direct contact with blood, mucous, stools or nasal discharge (Manson & Bahr, 1987). Occasionally infection may occur accidentally in the laboratory (Terry, 1949; Manson & Bahr, 1987), or experimentally through injection of L. donovani intradermally that can produce nodules containing amastigotes (Manson & Bahr, 1962, 1987). Congenital transmission has been reported (Manson & Bahr, 1987) as well as sexual transmission (Symmers, 1960; Manson & Bahr, 1987).
1.8. Immunology:

*Leishmania* a mastigotes produce variable effects on the elements of the immune system (WHO, 1990) Leading to variable host responses and producing a wide spectrum of disease (Manson & Bahr, 1995).

Infection with *Leishmania donovani* results in the development of organ-specific immunity in the two main target tissues of infection, the spleen and the liver. The liver is the site of an acute resolving infection associated with the development of inflammatory granulomas around infected Kupffer cells, and resistance to reinfection. Paradoxically, the spleen is an initial site for the generation of cell-mediated immune responses, but ultimately becomes a site of parasite persistence with associated immunopathological changes (Stanley, Engwerda2007).

Immunity against *leishmania* parasite is mediated by cellular mechanisms (Ghalib, 1993). Antibodies have little if any protective role against this organism (Probst et al., 2001); thus making the strategies of the host defense depend on the sequence of the reactions inside the macrophage.

The organism protects itself by an electron dense material, thus it resists macrophage killing by oxygen dependent or non oxygen dependent mechanism due to oxidative metabolites that interfere with the activity of the lysosomal enzymes. Therefore macrophage microbiidal activity is reduced (Hoover et al., 1985).

The parasite produces modulation of the T-cell response. There is marked depression of the cell-mediated immunity, depressed delayed type hypersensitivity and low numbers of T-lymphocytes in active VL cases, which are reversible with successful treatment (Aikat et al., 1979, Ghose et al., 1979). When CD4 T-cell is activated, the outcome depends on the balance between T.hl/T. h2 responses (Sundar et al., 1997; Probst et al., 2001). This balance is thought to be genetically
determined which is important in the pathogenicity of the disease. Resistance and elimination of the parasite is in favour of the response which mediates the production of INF.λ and IL-2. Th2 response which involves the secretion of IL-4, IL-5, IL-6 and IL-10 is associated with disease susceptibility. IL-10 down regulates the Th1 response (Ghalib et al., 1993). IL-15 is not functioning as a universal NK cell priming signal and that IL-18 contributes to the NK cell response in visceral leishmaniasis (Haeberein et al. 2010).

Assessment of cell mediated immunity can be done by either in vitro or in vivo tests. The in vitro tests are lymphocyte proliferative, response and phenotypic distribution of the peripheral mononuclear cells (PMNC). Usually there is failure of lymphocytes to respond to leishmanial Ag as well as mitogens, phytohaemagglutinin and concavalin A, which are reversible with successful treatment (Ghose et al., 1979). Peripheral mononuclear cells fail to respond to leishmanial antigen, in active KA, as well as PKDL (El-Hassan et al., 1992), as compared to the cutaneous form. The response is specific to leishmanial antigen as it reverts to normal with commencement of therapy in vivo.

Regarding the humoral immune responses there is marked increase immunoglobulin G (IgG) compared to immunoglobulin M (IgM) (Manson & Bahr, 1987; Mure's, 1997). IgGl and IgG3 (sub-classes of IgG) are very specific and correlated with the disease activity as they decrease after treatment (EL-Assad et al., 1994).

1.9. Pathology and pathogenicity of Kala-azar:

After the host is bitten by an infected sandfly, the parasites disseminate in the blood stream and enter macrophages of the spleen, liver, bone marrow, lymph-nodes, skin and small intestine. The promastigotes immediately lose their flagella and change their shape into the amastigote forms which attach themselves to the membrane of the macrophage and invade it. They spread from local lymph nodes
through the blood to different organs e.g. liver, spleen, bone marrow and lymph glands, rarely the intestine, adrenals, kidneys and lungs. A leishmanoma is formed in the skin after injection of the mammalian host with the promastigotes which may pass unrecognized.

The pathology of leishmaniasis varies and is determined by factors such as the environment, the insect vector, and parasite and host genetics. (Sakthianandswaran et al., 2009)

Usually there is anaemia which is commonly normocytic normochromic, sometimes thrombocytopenia and leucopenia occur which predispose the patient to secondary infection and haemorrhagic manifestations. (Nandy et al., 1998) reported a case presenting with renal haemorrhage. Pancytopenia might result from hypersplenism or bone marrow involvement.

The skin is commonly involved in kala-azar. In fatal cases all the skin layers are loaded with the parasite. In post kala-azar dermal leishmaniasis (PKDL), some of the parasites survive for a long time multiplying slowly in the skin. Other affected sites include the mucosa of the nasopharynx and oral cavity (El Hassan et al., 1995), which make the diagnosis of Kalazar difficult. PKDL patients may be an important source of infection in VL transmission (Zijistra & El Hassn, 2001).

Histocytic proliferation in these organs produces enlargement with atrophy or replacement of the normal tissue, therefore kala-azar is a systemic granulomatous disease of the reticulo-endothelial system (Manson & Bahr, 1987).

1.10. Clinical Presentation:

The natural history of the disease is progressive and it is fatal unless treated. The incubation period is variable, normally between 3-6 months (Hommel & Ashford, 1983). It can be as short as 10 days and as long as 10 years. The disease can affect younger age groups especially in the Mediterranean area (Less than 5 years) and East Africa (between 5-9 years). Males are more affected than
females with a ratio of 4:1 (Manson & Bahr., 1987). The disease can be endemic, sporadic or epidemic (WHO, 1990). The disease can be sub-clinical or asymptomatic (Napier., 1946; Manson & Bahr., 1987). The disease can presents with 3 clinical forms:

1. A primary leishmanoma which is a small papule appearing 4-6 months before the onset of the disease or may go unnoticed (Manson & Bahr., 1987).
2. An acute onset occurs either in sporadic or epidemic forms in non-immune people visiting an endemic area.
3. Chronic form which is the most common presentation in 3 endemic areas.

The patient presents with general ill health and cachexia (Cole, 1944 and Manson & Bahr, 1987). Fever is almost invariable, it can be high remittent with double peaks and associated with drenching sweating, but no rigors (Manson & Bahr, 1987); however, it can pass unnoticed by the patient. It may be intermittent or continuous in the established disease and may be absent in some cases (Napier, 1946). Fever is accompanied by progressive splenomegaly which may be very huge in size, reaching the right iliac fossa within a few days (Manson & Bhar, 1987). In a few cases the spleen is not palpable. Hepatomegaly is not to the same degree as splenomegaly. There may be cirrhosis, ascites or jaundice as a late complication of the disease. Diarrhoea may be one of the presenting symptoms due to gastro intestinal involvement, as well as epistaxis. Hyperpigmentation involves the hands, feet and abdomen (Napier, 1946). The lymph nodes are enlarged, especially in African kala azar.

There may be mild cough and proteinuria, (Manson & Bahr, 1987) and oedema (Napier, 1946). Amyloidosis is a recognized complication and may regress with cure. Manson & Bahr, (1987) reported a patient who presented with tremors. Other neurological involvements like delerium and confusional states have also
been reported (Manson & Bahr, 1987). Retinal haemorrhage is one of the presenting features of the disease.

Mucosal and mucocutaneous involvement occurs sporadically in the Sudan and Eastern Africa. Complications are important causes of death, these include: tuberculosis, lobar pneumonia, dysentery, liver cirrhosis and cancrum oris (Manson & Bahr, 1987).

**1.11. Hematological Features of VL:**

Anaemia is invariable, especially in children and it is usually normocytic normochromic (Zijistra & El Hassan, 2001), it may be due to hyper-splenism, auto-immunity with haemolysis or due to ineffective erythropoiesis. Leukopenia is marked and late in the disease. The total white blood cell count (TWBC) may reach levels below 2000/µl, and there may even be agranulocytosis in fatal cases.

Thrombocytopenia and pancytopenia were reported above. The erythrocyte sedimentation rate (ESR) may reach a very high level.

The haematological manifestations were reviewed in 94 patients (55 males and 39 females) with visceral leishmaniasis by (Nasir, A.M. et al 1995) in Saudi Arabia. They found that all patients had splenomegaly and were anaemic, while (73) were neutropenic and (56%) thrombocytopenic. Coagulation abnormalities were encountered in 10(11 per cent) patients; in four patients this was associated with disseminated intravascular coagulopathy. Bone marrow was hypercellular in (90%), normocellular in (5%), and hypocellular in (4%). Also variable degrees of erythrophagocytosis and leukophagocytosis were noted with preponderance of histiocytes (46%) and granulomatous formation (25%). Low haemosiderin content in the bone marrow was noted, which together with the finding of high serum ferritin is consistent with anaemia of chronic inflammation.
1-12: Biochemical changes of Visceral Leishmaniasis:

Renal dysfunction is an important feature of this disease; it is associated with important morbidity and can increase mortality. (Daher et al., 2008)

Renal changes consisted of the following: Glomerular lesions as segmental collapse of capillary loops, congestion of capillaries filled by Leishmanias, capsular synechiae, focal and segmental areas of glomerular sclerosis, focal thickening of basement membranes, parasite invasion of mesangial axes (cells and matrix) and focal mesangial and epithelial cell hyperplasia with initial crescent formation. Immunohystochemical examination was negative for immunoglobulins and light chains. Tubulo-interstitial damage as acute necrotizing tubulitis, tubular necrosis and hyaline casts, focal infiltration of CD68 and CD3 cells, and Leishmania inside of histiocytes in peritubular areas (Pintado et al., 2001). Blood vessel changes: as focal leukocytoclastic vasculitis of vasa recta and free Leishmanias in the capillary lumina. Acute glomerulonephritis (GN), proliferative GN, collapsing focal segmental glomerulosclerosis, acute interstitial nephritis and tubular cell necrosis and tubulitis have all been described in patients with Leishmaniasis (Pintado et al., 2001). In study carried about the disturbances in function of the tubules in human Kala-azar the principal alterations were hyponatremia 94.6%, hypokalemia 26%, hypocloremia 27.2%, hypocalcemia 32%, hypomagnesemia 41.8%, hypouricemia 14.3%. Increased urinary excretion fraction were: sodium 15%, potassium 26%, chloride 33.3%, calcium 32%, inorganic phosphate 27.2%, magnesium 100% (hypermagnesiuria), uric acid 44%. Glucosuria was found in one third of patients. There was evidence of renal proximal tubular damage with alterations in the reabsorption of proteins and light chains with characteristics of a tubular proteinuria, Disturbances of tubular reabsorption of uric acid, calcium, phosphate, glucose and magnesium were also observe (Agenor Araujo Lima et al., 2009) The renal damage in patient was
mediated through different mechanisms. Tubular necrosis was secondary to ischaemia, due to small vessel obliteration by Leishmanias, and to haemolysis. Necrotizing tubulitis was due to direct invasion by the parasites and by inflammatory reaction. There was a diffuse invasion of renal structures by Leishmania (Pintado et al., 2001).

In study carried in the Department of Nephrology and Kala-azar unit of Institute of Medical Sciences, Banaras Hindu University, Varanasi, India between April 2002 to May 2004, the renal involvement was documented in (15%) cases. The spectrum of renal disease included; proteinuria in the range of 1-2gm/day (15%), abnormal urinary sediment (4%), and acute renal failure (15%) of cases. Dialytic support was not needed (Prakash et al., 2007)

In Italy, (Rollino et al., 2003) reported a renal failure in 28-years old VL/HIV infected Nigerian woman who had been in Italy for 8 months, attended the hospital because of epistaxis, confusion and anuria. Her temperature was 36.3°C, O2 saturation 98%, blood pressure 120/70 mmHg and cardiac pulse 125/min. Her physical examination was unremarkable, except for abdominal tenderness. Laboratory investigations disclosed: HIV positivity, serum creatinine 20.7 mg/dl, Na 140 mEq/l, K 7.5 mEq/l, Hb 7.9 g/dl, MCV 74 fl, WBC 4050/mm3, platelets 557 000/mm3, AST 424 U/l, ALT 41 U/l, bilirubin 3.6 mg/dl (3.4 mg/dl indirect), CPK 55 U/l, LDH 8096 U/l. There for dialysis was initiated. Light microscopy examination evidenced striking diffusion of Leishmanias in phagocytic cells and extracellular presence in vascular lumina and connective tissues (kidney, liver, nasopharynx, lung, skin, uterus, ovary, lymph nodes, bone marrow, heart and spleen).

They concluded that, Leishmania behaves as an opportunistic infection in HIV-infected individuals. It should be suspected in African people in a case of
acute renal failure, when no other causes can be advocated. ARF can be due to direct invasion of parenchyma and to tubulo-interstitial and glomerular structures involvement (Pintado et.al, 2001).

Also A 29-year-old full-term pregnant woman from a rural area of Iran, the Pars province, presented with fever of 2 months’ duration. On physical examination, the only positive finding was a high temperature of 39.5°C. Due to a protruded abdomen, the liver and spleen could not be palpated. The clinical workup determined only the presence of anemia. On the second day after admission, the patient and fetus died. Autopsy revealed surprisingly numerous Leishman bodies in most organs, including the liver, spleen, bone marrow, adrenal, placenta, and kidney. In addition to increased numbers of Leishman bodies, the autopsy also revealed extensive centrilobular necrosis of the liver, severe congestion and white pulp depletion of the spleen, severe pulmonary congestion and edema, bilateral hemorrhagic necrosis of adrenals, and mesangioproliferative glomerulonephritis. They report the presence of typical Leishman bodies inside the mesangial cells of the glomerulus (Daneshbod et. al,1972).

Also in Sudan LFT done for VL patients during an outbreak in white Nile province and they report presence of jaundice, hypoalbuminaemia, hyperglobulinaemia and elevation of liver enzymes (AST, ALT and ALP) (Mustafa 1995).

1.13. Diagnosis of VL:

There are three main diagnostic approaches for the confirmation of Kalazar. These include the parasitological, immunological and DNA based detection methods.
1.13.1. Parasitological diagnosis:

1.13.1.1. Direct microscopy:

The definitive and routine diagnosis of VL leishmaniasis relies on microscopic detection of leishman donovan bodies in Giemsa stained smears of lymph node, bone marrow or splenic aspirates (Evans, 1989). These methods are relatively simple and cheap but there is no possibility to distinguish between *Leishmania* amastigotes spp (Evans, 1989); while some of them lack sensitivity, others are invasive. Although splenic aspiration is generally accepted as the most sensitive method and can detect more than 90% of cases (Manson & Bahr, 1987), it is contraindicated because bleeding complications are feared. At least three separate studies showed a sensitivity of at least 94% for splenic puncture in VL patients (Siddig *et al*., 1988; Zijistra *et al*., 1992). Bone marrow aspiration unlike splenic aspiration or lymph node aspiration is often resented by the patient because it is painful especially in children. Microscopy of lymph node aspirates is the most commonly used procedure in endemic areas of Kala-azar in the Sudan as it is a safe and simple procedure (Siddig *et al*., 1988). However, a variable sensitivity was reported ranging from 58.3% (Zijistra *et al*., 1992) to 100% (Kirk and Satti, 1940). Liver aspiration is used infrequently in the diagnosis of VL (Zijistra *et al*., 1992). In HIV/ *Leishmania* co-infected patients the amastigotes are found in the peripheral blood in 50% of cases and may be found in unusual location such as the lungs, larynx, gastrointestinal tract and C.S.F. (Manson & Bahr., 1987).

1.13.1.2. In vitro culture of visceral leishmania:

Culture of *Leishmania* parasites for diagnosis of leishmaniasis has been used since the beginning of 20th century. Several culture media have been used including NNN and various semi-synthetic media, supplemented with blood or serum (Evans, 1989). For primary isolation of *Leishmania* parasites, small inocula of clinical material (lymph node, bone marrow, spleen aspirates) is inoculated
under aseptic condition into the liquid phase of blood agar biphasic media. The inoculated media are incubated at 24±2°C for 7-20 days using cooled incubator. Growth in culture tubes was detected by turbidity in the liquid phase of the media and confirmed microscopically by presence of motile promastigotes in smear preparations (WHO, 1996).

Culture media for mass cultivation and primary isolation of old and new world forms of *Leishmania* spp include blood agar biphasic medium e.g NNN medium, sloppy Evans medium, semi solid Lockes blood agar, Evans modified Tobies medium Usmaru (Difco BA) MEM: FCS: EBLB medium and Schneider's Drosophila medium (Evans., 1989). The organisms from patients with VL can be very difficult to cultivate (Evans, 1989). Therefore it is wise whenever possible to inoculate experimental animal as well. In attempt to cultivate *Leishmania*, it is advisable to use blood agar biphasic media preferably e.g NNN or USAMRU or modified Tobies medium but even when the initial isolation is successful the organism may die when sub-cultured. This seems to be especially common when the initial isolation has been into rich media such as USMARU or modified Tobies. Often this can be overcome if sub-cultures are made into less nutritionally rich media such as NNN or one of the semi solid media like sloppy Evans and semi solid Locke's blood agar (Evans., 1989& WHO., 1990). The variations of these media lead to efficacy in the transformation of amastigotes to promastigotes and in the growth rate of promastigotes. While being easy to carry out, *Leishmania* culture needs some expertise and sterile conditions during collection and processing of material from patients lesion or organs, and for sub culturing (Dedet et al., 1999).

The purposes of culture of the genus leishmania include: accurate identification of the organism, to obtain a rich yield of the organism to be used as antigen in immunological diagnosis for teaching purposes, as a source for
inoculating susceptible experimental animals, for in vitro screening of drugs and for investigation of the physiology of the pathogens (Cheesbrough, 1995). Initial isolation is the process by which *leishmania* organisms are removed from their host and transferred either to an experimental animal (in vivo isolation) eg hamster or into culture media (in vitro isolation) so that multiplication and bulk cultivation of *leishmania* is necessary for isoenzyme identification methods which require relatively a large number of promastigotes. The choice of isolation methods depends on the immediate circumstances and to some extent on the technical capabilities and experience of the staff. In-vitro isolation offers certain advantages over the in vivo methods as cultures become more rapidly positive after as a short time as 5-7 days in contrast to a week or a month required for a lesion to appear in an animal after inoculation the culture materials are less expensive and cultured organisms can be cryopreserved thus reducing the time and personnel required for its maintenance. The disadvantages of in-vitro culture are that some strains of *leishmania donovani* are extremely difficult to attain in the field (Evans, 1989). In vitro cultivation is an efficient method for the parasitological diagnosis of leishmaniasis and for distinguishing between various strains causing leishmaniasis. The most difficult part is usually the initial establishment of the organism in culture when the amastigotes have to transform to promastigotes to begin multiplication and this step needs skills and technical capabilities as well as abilities in preparation specification and sterility during inoculation of the clinical samples into the different types of media.

1.13.1.3. Animal inoculation (in vivo propagation):

Hamster is considered to be a suitable model for propagation of *Leishmania* (Evans, 1989). The animal is inoculated intradermally or subcutaneously as well as in the nose and hind feet. A positive reaction is identified by finding an indurations or an ulcer at the injection site. The presence of amastigotes can be verified by the
examination of stained smears. Other animals like guinea pigs and inbred mice can be used (WHO, 1996). Usually laboratory animal inoculation is slower than culture methods. It takes 3-4 weeks for lesions to appear in contrast to the culture which takes 10-15 days for the development of the promastigotes (Evans, 1989). Previous investigations by (Satti et al 1958) also demonstrated that the grey monkey (Cercopithecus sebaezeis) and the red monkey (Cercopithecns ruler) has successfully became infected with L. donovani parasites. The method however, was criticized for being time consuming, laborious and unsuitable for routine diagnosis.

1.13.2. Immunological diagnosis:

Immunodiagnosis is of paramount importance in the diagnosis, follow up and control of infections. It can be divided into two parts: one which detects and measures antibodies in the serum and the other which can demonstrate or detect the cell mediated response (Bray, 1985; Lakshams, 1985). Immune-diagnosis is needed for rapid and reliable detection of early cases especially in the field when the parasite load is scanty and may not be revealed in smears. However it’s main problem is cross reactions. At present several immunological techniques based on cellular or humoral responses are widely used for the diagnosis of VL.

1.13.2.1. Leishmanin skin test (L.S.T or Montenegro test):

This test is a delayed type hypersensitivity reaction to Leishmania analogous to tuberculin and lepromin tests (Cheesbrough, 1995). The antigen consists of a suspension of killed promastigotes. The test is performed by intradermal injection of 0.1 ml phenolized antigen into one forearm and 0.1 ml phenol saline into the other forearm (Bray, 1976). The test is usually read after 48-72 hours (Zuckerman, 1975 Bray, 1976) A positive reaction is indicated by a raised induration more than 5mm accompanied by erythema (Manson & Bahar, 1987). The test is not of diagnostic value in VL, but can help in epidemiological surveys.
of past and subclinical infection in endemic areas as well as in the exclusion of active VL (Manson & Bahar, 1987, Zijistra et al 1993 and El Hassan et al 1993) showed that a positive LST indicates that a treated VL patient has developed cell mediated immunity to *Leishmania*. LST is positive in active and past infection of cutaneous leishmaniasis for life (Manson & Bahar, 1987).

1.13.2.2. Non Specific Tests:

The nonspecific reactions depend upon the increase of the immunoglobulin seen in Kalazar. It can be demonstrated by flocculation or precipitation (Zuckerman, 1975). The best examples are:

1.13.2.2a Formal gel test (Napier aldehyde test):

This test is performed by mixing 1 ml of serum with 2 drops of 30% formalin. The reaction is read after 20 minutes. A positive reaction is indicated by whitening and jelly appearance which indicate hyperglobulinaemia (Ghose, 1980). The test is positive after 1-2 months of the infection and becomes negative after 6 months of cure. It is simple easy to perform and cheaper therefore it is suitable for sero-epidemiological survey (Jalil et al., 1992). Sensitivity of this test is 62.7% and specificity is 93.2% (R. Kumar et al., 2006). It has a diagnostic value in chronic cases of kala-azar (Sen Gupta et al., 1969). The main disadvantage is the cross reactivity with other diseases that cause hyperglobulinaemia e.g leprosy, TB, trypanosomiasis and schistosomiasis.

1.13.2.2b Antimony (chopra test) or urea stibamine test:

This test is used in India. The serum is diluted 1 in 10 with distilled water and placed in a narrow bore test tube then 4% urea stibamine solutions added and mixed gently. A positive reaction is indicated by flocculent precipitate after 10-15 minutes. It is heavy in strong positive reaction (Manson & Bahar, 1987).
1.13.3. **Specific tests:**

Various tests have been applied to detect specific anti leishmanial antibodies. These include DAT, ELISA, IFAT, CFT (Bray, 1985). Recently DAT, IFAT, ELISA and WB were considered as important diagnostic tools.

1.13.3.1. **Complement fixation test (CFT):**

Complement fixing antibodies appear in 95% of cases from the third week of infection and disappear within 6 months of cure (Manson & Bahar, 1987). The antigen is prepared from *L. donovani* promastigotes (Manson & Bahar, 1987). A titer of 1/10 is suggestive and a titer of 1/40 is diagnostic (Manson & Bahar, 1987). The problem with CFT is that it can give false positive results with tuberculosis, leprosy and less commonly with malaria. Persistence of a low titer is a clue to relapse.

1.13.3.2. **Indirect haemagglutination test (IHT):**

The haemagglutination test follows the same course as CFT (Manson & Bahar, 1987). The antigen is in a soluble form prepared from promastigotes adsorbed into tanned sheep erythrocytes and latex particles. It is a sensitive and specific test (Manson & Bahr, 1987). A titer of 1/200 and over is diagnostic (Bray, 1976).

1.13.3.3. **Indirect fluorescent antibody test (IFAT):**

This is a sensitive and specific test in diagnosing VL (Manson & Bahr, 1987). However, other studies reported a sensitivity of 75% and a specificity of 93% as compared to ELISA (Ashford *et al.*, 1993).

The antigen is prepared either from promastigotes fixed with acetone or I, form a mastigotes isolated from an infected animal. It is particularly important in screening and detecting early cases of kala-azar (Manson & Bahar, 1987) as it becomes positive early in the disease and disappears within 6 months of cure.
Persistence of antibodies for more than 2 years is indicative of relapse and is also a measure of the response to treatment (Manson & Bahar, 1987).

The disadvantage of the test is its cross-reaction with T. cruzi (Shaw & Volter, 1964; Bray & lanson, 1965; Manson & Bahar, 1987) malaria, schistosomiasis and leprosy as well as typhoid and larva migrans infestation (Manson & Bahr, 1987). Another disadvantage is that the test needs a fluorescent microscope and it is not suitable for large scale epidemiological studies.

1.13.3.4. Enzyme linked immunosorbent assay (ELISA):

The use of enzyme linked assay with an antigen or an antibody was first described by (Engvall and Perlman 1971, 1972). They assessed the antibodies present in rabbit sera by using disposable polystyrene tubes and alkaline phosphate as a conjugate and when compared with RIA they showed that ELISA was sensitive. Since that time ELISA has been widely adopted in the serodiagnosis of parasitic diseases like malaria, trypanosomiasis, giardiasis, schistosomiasis, ect... (Voler et al., 1980).

The principle of the test is that the antigen is adsorbed to a solid phase (polystyrene surface) by physical adsorption to which is added the serum and the enzyme labeled anti-immunoglobulin that retains both the immunological and the enzyme activity. The intensity of the colour produced when the substrate specific for the enzyme is broken is proportional to the amount of the specific antibody present in the serum (Voler et al., 1980). The test was shown to have a sensitivity of 98.4% and specificity of 100%.

1.13.3.5. Immunoblotting (Western Blot WB):

The performance of Leishmania soluble antigens for VL diagnosis was improved through electrophoretic separation of individual reactive epitopes as in immunoblotting techniques (Jaffe & Zaiis, 1988). Incorporation of purified polypeptides of L. infantum in such a technique provided in addition to the high
potential for VL diagnosis also the means to study the evolution of antibody responses throughout the course of infection. The optimal sensitivity of immunoblotting techniques and in particular the Western Blot (WB) was further demonstrated in patients with VL/HIV co-infection. Nevertheless Hoerauf et al. (1992) demonstrated the capability of the immunoblot technique to differentiate kala-azar from lymphoproliferative and autoimmune diseases that could not be differentiated by ELISA and IFAT. However the technical and financial factors involved constituted significant obstacles for the applicability of immunoblotting techniques at the central laboratories of various VL endemic areas. In Sudan (Bucheton et al. 2003) analysed Leishmania-specific antibodies by enzyme-linked immunosorbent assay and immunoblotting. Immunoblot analysis detected antibodies to Leishmania in 80% of the healthy subjects and half of them harboured high immunoglobulin (Ig) G antibody levels, similar to those of VL patients. These antibodies belonged to the IgG1 and IgG3 subclasses but neither their respective levels nor the immunoblot recognition patterns were predictive of VL.

1.13.3.6. Direct agglutination test (DAT):

The direct agglutination test is a simple and feasible technique that has high sensitivity and specificity (Harith et al., 1986, 1988, Hommel, et al., 1997; Meredith et al., 1995). Because of these advantages it is one of the most widely used immunological tests that have been applied by several investigators in diagnosis and epidemiological studies. In this test the antigen preparation consists of the whole organism (promastigotes) and the serological response (mainly IgG) to surface borne antigens is measured. Nicloe and Manceaux in 1909 were the first to describe agglutination in the serum of patient with Leishmania while the first diagnostic application in the serum of patient with Leishmania of the agglutination test was performed by Row in 1931 (cited by Hommel et al., 1997).
specificity of the test was obtained by trypsinization of antigen. The DAT was modified by (Harith et al. 1986) who described formalin-fixed trypsinized promastigotes stained with coomassie brilliant blue and used as an antigen in a reusable V-well microtitre plates. The sensitivity and specificity of the DAT were further improved by testing the serum samples in serum diluent containing gelatin instead of fetal calf serum and 0.1 ml 2-mercaptoethanol (Harith et al., 1988). Cleaving agents other than trypsin e.g pronase lipase pancreatin or 2-mercaptoethanol were evaluated for further improvement of the test (Harith et al., 1995).

The test was recommended to be used widely in sero-epidemiological surveys. (EL Harith et al. 1996) showed that the DAT was valuable in differentiating PKDL from other skin conditions like leprosy.

According to (Hommel et al. 1997) the possible cross-reactivity with other pathogens including T brucei, T. cruzi and Mycobacteria Tuberculosis are the critical problem in the DAT. Andrade et al. (1987) showed that L. donvani DAT antigen could be used for the diagnosis of VL caused by L. chagasi in Brazil. (Harith et al. 1988) found cross- reactivity with serum samples from patients with African trypanosomaisis and toxoplasmosis.

These cross reactions were eliminated by the addition of 2-mercaptopethanol to the serum diluents. The decline of anti-leishmanial antibodies level in VL patients following treatment has been a subject for intensive research in the course of DAT evaluation. (Hailu 1990) found that significant level of anti leishmanial antibodies may be present up to 7 years after treatment. Like as most serological tests, DAT may not differentiate between active disease, sub clinical and pervious infections and this is a major draw back to the test (Hommel et al., 1997; Zijistra et al., 1991). Another major draw back to DAT is the limited stability of the aqueous antigen (Harith et al., 1988). Of due to that, a DAT based
on stable - freeze -dried antigen has been developed. High sensitivity (92%) and specificity (99.7%) of the freeze dried antigen were shown to be identical to that of aqueous antigen (Oskam et al., 1999). The freeze -dried antigen can be stored at ambient temperature. Due to these factors the test became one of the best available diagnostic tools for use in the field.

1.13.3.7. Latex agglutination test (LAT):

In search for a quick simple serodiagnostic test, many researchers sensitized the latex particle with Leishmania antigens. De Korte et al 1990) performed LAT sensitized by L. infantum soluble antigen for the detection of anti-Z., infantum antibodies in canine VL reservoir in southern France but they obtained a lower sensitivity for LAT in comparison to DAT. (Moody & El Safi 1996) performed the LAT (using L. donovani soluble antigen) in sera from Sudanese VL confirmed cases (n= 60), VL unconfirmed cases (n=48) but with positive DAT result and sera from healthy non endemic controls (n=75) they reported a sensitivity of 88% and specificity of 100%. They concluded that the LAT is a simple and practical serological technique for the diagnosis of VL particularly at dispensary level but they recommended further evaluation of LAT using fresh sera. In study done for evaluation and development of latex agglutination test ; using plain latex, carboxylated latex and magnetic beads. They found that the sensitivity was 95% and specificity of 90%( Ahmed abdAllah thesis)

1.13.3.8. Latex agglutination test (Katex) for detection of urinary antigen:

Katex is a new latex agglutination test for the detection of leishmanial antigen (Ag) in the urine of patients with VL (Attar et al., 2001; Hommel et al., 2001;). The detected Ag which is a heat-stable, precipitates with acetone and ethanol but not Tri acidic acid, is sensitive to periodate and acid hydrolysis but not to Pronase E, Lipase,or neuraminidase. The Ag is a low molecular weight glycol conjugate that can be extracted by phenol water, the molecular weight of urinary
Ag is 5-20 KDA was detected in the urine of patients from Nepal, Sudan, Brazil, Yemen and Spain and experimentally infected animals (Sarkari et al., 2002). No low molecular weight antigen was detected in the urine of patients with malaria, schistosomiasis, or diseases such as typhoid and brucellosis (Sakari et al., 2002). The antigen is detectable in both the promastigote and amastigote stages of the parasite. Monoclonal antibodies (mAbs) against Leishmania glycoconjugates strongly react with this molecule, so that the detected antigen is highly specific and diagnostic for VL.

The test is based on the adsorption of anti-leishmanial antibody to latex particles; this reagent agglutinates within two minutes when mixed with urine from an infected host. To obtain adequate specificity urine can be boiled before performing the test. As antigen detection tests the Katex would in principle provide better means for diagnosis since antigen level are expected to broadly correlate with the parasite load. Antigen detection systems are also ideal alternative to the antibody detection systems in immuno compromised patients. Katex is simple test and easy to perform. It does not require specialized equipment and easy to interpret. The test is 100% specific and 80% sensitive with parasitologically confirmed cases from Brazil, Sudan, Yemen and Nepal (Attar et al., 2001).

1.13.3.9. rk39 strip test:
A test based on a 39-amino-acid-repeat recombinant leishmanial antigen from Leishmania chagasi (rK39) has been introduced into an enzyme-linked immunosorbent assay (ELISA) and, later, an immunochromatographic strip tes for the diagnosis of VL (Zijlstra, et al. 1998). The rK39 strip test is reported to be simple, sensitive, specific, non-invasive and economical test (Singh et al., 2009)

The test seems an ideal qualitative test for the diagnosis of kala-azar. But for sero-epidemiological studies the test may be used with other parameters.
Alternatively a quantitative ELISA using rK39 antigen may be used. (singh et al., 2009)

1.13.4. DNA based detection methods:

In recent years several molecular biological techniques have been developed for the sensitive detection and identification of pathogens (Denissereno et al., 2003). The main approaches to nucleic acid based detection are: (1) by hybridization using DNA probes and (2) amplification techniques including NASBA, RT.PCR for the detection of RNA and the PCR for detection of DNA. In one study, none of patients treated for VL who tested negative by PCR with lymph node tissue relapsed or developed PKDL, while more than half of patients who tested positive by PCR with lymph node tissue either relapsed or developed PKDL after apparent cure of disease following supervised treatment (Osman et al., 1995).

A group of Sudanese researchers who used PCR for detection of reservoir hosts headed by (EL Hassan et al 1993) detected Leishmania DNA in Arvicanthns niloticis.

The availability of DNA based diagnostic methods has allowed more sensitive, rapid detection and characterization of microorganisms. The association of hybridization with the PCR has solved relatively limited sensitivity of hybridization and has been used with efficacy for the diagnosis of genetic and infectious diseases including protozoan parasites (Ashford et al., 1995).

(Scharfer et al 1995) examined the sensitivity of PCR for detection of parasite DNA before and after diagnosis and treatment. They found that PCR detects sub clinical VL cases; i.e. have detectable circulating DNA in their blood. They showed that L. donovani DNA can be detected in the blood despite negative serological tests, and absence splenomegaly. In addition, they found that parasite DNA can be found in blood dried on filter paper long before a parasitological
diagnosis of VL is made of helps to provide them to center involved in clinical trials and parasite characterization (WHO, 1996).

1.14. Treatment:

Pentavalent antimonial drugs have been used in the treatment of VL since 1916 (Manson & Bahr, 1987). They are the first line of VL treatment. They are available in form of sodium antimony gluconate (Pentostam) and meglumine antimonate (Glucantime) at the rate of 85mg/ml pentostam available in vials (50ml) which contain 600mg. It can be given intramuscularly or intravenously at a daily dose of 20mg/kg body weight for about 30 days. Antimony compounds act against *Leishmania* parasites by inhibiting purine nuclear and macro cellular synthesis (Bracyson, 1987). Renal function has to be assessed before and during drug treatment because 60-80% of the drug is renally excreted. It is also cardiotoxic and can produce fatal arrhythmias in high dosage especially during the second week after commencing treatment (Chowdhury *et al*., 1998). Most of the patients respond well to antimonial compounds. Other drugs used include pentamidine but it has side-effects. Amphotericin B kills the parasite intra and extracellularly but is nephrotoxic. The new formulation of Amphotericin with lipid (Ambisome) fortunately devoid of the toxicity of Amphotericin B (Hoiumer *et al*., 1998) and can be used for VL patients who are not responding to the treatment of kala-azar patients and they enhance intracellular killing of the parasites. A single infusion of liposomal amphotericin B was not inferior to and was less expensive than conventional therapy with amphotericin B deoxycholate. (Sundar *et al*., 2010) INF-λ has been used for the treatment of Kala-azar patients and it also enhanced intracellular killing of *Leishmania* and when used in combination with antimonial compounds in refractory VL cases gave satisfactory results (Murray *et al*., 1988). All the current drugs for kala-azar have
draw backs. They are administered by injection or infusion and require the patients to be hospitalized. Miltefosine is expected to be the first oral treatment of kala-azar in Bihar-India (TDR News, 1999).

2. HIV/AIDS:

Human immunodeficiency virus (HIV) types derived from primates (Lentiviruses) are the etiologic agents of AIDS (Geo & Butel, 1998). Scientists identified a type of chimpanzee in West Africa as the source of HIV infection in humans. The virus most likely jumped to humans when humans hunted these chimpanzees for meat and came into contact with their infected blood. Over several years, the virus slowly spread across Africa and later into other parts of the world (CDC.2009)

The illness was described for the first time in 1981 by Luc Montagnier of France and Robert Gallo of the United States. AIDS etiologic agent HIV-1 was isolated by the end of 1983. Since then AIDS has become a worldwide epidemic expanding in scope and magnitude.

About 54,862,417 persons were estimated in November 2003 to be infected worldwide, 30% of them were in South Africa (Kahn et al., 1998). Once they contract the disease, individuals remain infected for life if left untreated. The vast majority of HIV infected individuals develop fatal opportunistic infections such as tuberculosis as a result of HIV-induced deficiencies in the immune system. AIDS is one of the most important public health problems world-wide at the start of the 21st century (How et al., 1998).

2.1. Virus morphology & structure:

The virion of the HIV is spherical 80-100µm in diameter, of cylindrical core. The genome is single strand RNA, linear, positive sense 9-10 kilobases in length, diploid and contains up to six additional replication genes (Geo & Butel, 2001). The mature virion has three morphologic components:
- An outer envelope made up of lipid bilayer membrane containing virus specific glycoprotein spikes.

- An internal protein capsid, within the capsid, a nucleocapsid and two virally encoded enzymes (Reverse transcriptase and integrase).

In addition, unique cellular tRNAs are associated with each segment of the viral genome.

The Lentivirinae subfamily to which HIV belongs is characterized by a distinctive nucleocapsid core viewed in electron microscope as a bar or truncated, cone-shaped nucleocapsid.

HIV has three major genes coding for structural proteins. These are gag, pol and env. The gag-derived proteins make up the viral capsid p24, i.e. a 24 kilodalton protein, the nucleocapsid protein (p17) and a matrix protein.

The pol gene code for the virus enzymatically active proteins most important of which is reverse transcriptase, which performs the unique reverse transcription of the viral RNA into double stranded DNA.

Also pol encodes a specific viral protease. This enzyme cleaves gag and gag-pol derived protein into functional pieces. The third structural gene, env, stands for "envelope". The proteins derived from env are a surface (gp 120) and a transmembrane protein TM (gp 41) (Geo & Butel, 2001).

The TM (gp41) env product contains both a transmembrane domain that anchors the glycoprotein in the viral envelope and a fusion domain that facilitates viral penetration into target cells. The divergence in the envelope of HIV complicates efforts to develop an-effective vaccine for AIDS (Volk et al., 1996).

2.2. Target cells and mechanism of infection:

HIV primarily infects cells with CD4 cell surface receptor molecules, using them to gain entry. HIV use CD4 molecule as receptor, which is expressed on macrophages and T lymphocyte. HIV may use galactosyl ceramide as receptor in
place of CD\textsubscript{4} in neural cells. Second important receptor is necessary for HIV-1 to gain entry to cells is chemokine receptors, chemokines are soluble factor with chemo-attractant and cytokine properties. CCR5, the receptor for chemokines RANTES, MIP.1\textalpha, and MIP.1B, is the receptor for macrophage-tropic strains of HIV-1. In contrast, CXCR4, the receptor for chemokine SDF-1, is the necessary co-receptor for lymphocyte-tropic strains of HIV-1 (Buseyne \textit{et al.}, 1998). The presumed mechanism is that HIV needs to bind to both a T cell CD4 receptor and to a co-receptor (Buseyne \textit{et al.}, 1998). The viruses first bind to CD4 and then to the second receptor, this interaction causes conformational change in the viral envelope activating the gp41 fusion peptide and triggering membrane fusion. There is evidence that individuals who possess homozygous deletion in CCR5 may be protected from infection by HIV-1. Genetic variation in the chemokine co-receptor molecule results in less effective HIV binding and thus slows HIV replication.

Studies show that a homozygous deletion (delta-32) in the gene coding for the chemokine receptor CCR5 significantly lowers, but does not eliminate completely, the risk of HIV infection. (Liu \textit{et al.}, 1996; Huang \textit{et al.}, 1996; Samson \textit{et al.}, 1996; Dean \textit{et al.}, 1996; Theodorou \textit{et al.}, 1997; Biti \textit{et al.}, 1997). A second genetic variant (CCR2-64I) in the gene coding for the CCR2 receptor is not protective against HIV infection, but is associated with slower progression to AIDS by 2 to 3 years in heterozygous or homozygous individuals. It occurs in roughly 2\% of African Americans, Caucasians, Asians, and Hispanics (Smith \textit{et al.}, 1997; Kostrikis \textit{et al.}, 1998). A third genetic variant (SDF1-3'A) affects the regulatory region of a gene coding for the chemokine SDF-1, which is the ligand (the normal binding molecule) for chemokine receptor CXCR4 (Winkler \textit{et al.}, 1998).
2.3. Replication Cycle:
The replication cycle of HIV-1 include the following:

A. Virus entry:

   The first step is adherence of HIV by gp 120 to host cells, (Capon & Ward, 1991) that contain in their surface, the CD4 membrane antigen as receptor, which is found on T.lymphocyte (Bhat et al., 1993). These cells have specific chemokine receptors, (Alkhatib et al., 1996) known as co-receptor, (Deng et al., 1996) such as CCR5 or HIV-1R5, which is present in the early stages of the disease. (Alkhatib et al., 1996; Deng et al., 1996; Dragic et al., 1996), and CXCR4 or HIV-1X4 which is seen during late stages of the disease (Chose et al., 1996; Doranze et al., 1996 ).

   The second steps after attachment to host cells surface is penetration of HIV-1 virion into host cells. The gp41 is responsible for this (Stein et al., 1987; McClure et al., 1988, 1990).

B. Replication: (Reverse transcription &Integration)

   After internalization, the HIV virion is uncoated and its RNA is copied and generates a single stranded DNA copy (Katz & Shalka, 1990). This reaction is mediated by the Reverse Transcriptase, an RNA-and DNA-dependent DNA polymerase and RNase H, which degrades the RNA component of RNA-DNA hybrid molecules (Katz & Shalka., 1990). Then the second DNA strain is formed, also mediated by Reverse Transcriptase, which produce large amount of DNA (Shih et al., 1988). The recently formed double stranded DNA penetrates the nucleus as part of the pre integration complex reaction. The nuclear viral complexes serve as the machines that integrate viral DNA into host cell chromosomal DNA to form provirus. This step is dependent on the activity of the viral Integrate protein (Pryciak & Varmus, 1992).
C. Proviral DNA:
Following the integration of the recently synthesized viral DNA into the host DNA, this proviral DNA start to encode viral protein formation. Initially, viral mRNA is transcribed by proviral DNA and this mRNA mediated by host cell RNA polymerase, initiates synthesis of large poly proteins encodes the regulatory and structural viral proteins (Jacks et al., 1988; Parkin et al., 1992; Chamorro et al., 1992).

D. Budding:
After formation of HIV structure, the virion assembles into the cytoplasm. HIV is released from the cell by budding. It acquires it’s lipid membranes from the host cell membranes after this budding, the protease enzyme cleaves the virion poly protein leading to maturation of the infections viral particle. In the productive growth cycle the host cell is destroyed (Earl et al., 1991).

2.4. Pathogenesis:
The typical course of untreated HIV infection spans about a decade. The stages include the primary infection, dissemination of virus to lymphoid organs, clinical latency, elevated HIV expression, clinical disease and death (Geo, 1998). The duration between primary infection and progression to clinical disease averages about 10 years. Through untreated cases death usually occurs within 2 years after the onset of clinical symptoms.

Following primary infection, there is a 4-11 days period between mucosal infection and initial viraemia; viraemia is detectable for about 8-12 weeks, virus is widely disseminated trough out the body during this time and the lymphoid organs become seeded.

An acute mononucleosis-like syndrome develops in many patients (50-75%) 3-6 weeks after primary infection (Geo, 1998). There is significant drop in numbers of circulating CD₄ T cell at this early time. An immune response to HIV
occurs 1 week to 3 months after infection, plasma vireamia drops and levels of CD₄ cells rebound. However the immune response is unable to clear the infection completely and HIV infected cells persist in the lymph nodes.

This period of clinical latency may last for as long as 10 years. During this time there is a high level of ongoing viral replication. It's estimated that 10 billion HIV particles are produced and destroyed each day. The half life of the virus in plasma is about 6 hours and the virus life cycle (from the time of infection of a cell to the production of new progeny that infect the next cell) averages 2 – 6 days.

CD₄ T lymphocytes are the major targets responsible for virus production; appear to have similar turnover rates (Geo, 1998). Once infected, the half life of CD₄ T lymphocyte is about 1 – 6 days. Because of this viral proliferation and the inherent error rate of the HIV reverse transcriptase, it is estimated that every nucleotide of the HIV genome probably mutates on a daily bases.

HIV found in patients with late stage disease is usually much more virulent and cytopathic than the strains of virus found in early infection(Geo, 1998). Often a shift from monocyte or macrophage tropic (M-tropic) strains accompanies progression to AIDS.

The cardinal feature of HIV infection is depletion of T helper, inducer lymphocytes, as a result of the tropism of HIV for this population of lymphocytes, which express the CD₄ phenotypic marker on their surface (Geo, 1998). The CD₄ molecule is the major receptor for HIV; it has a high affinity for the viral envelope. The HIV co-receptor on lymphocytes is the CXCR4 chemokine receptor. Early in infection primary HIV isolates are M-tropic. However, all strains of HIV infect primary CD₄ T cells (but not immortalized T cell lines in vitro). As the infection progresses the dominant M-tropic viruses are replaced by T-tropic viruses.
Laboratory adaptation of these primary isolates in immortalized T cell lines, results in loss of ability to infect monocytes and macrophages.

The consequences of CD$_4$ T cell dysfunction caused by HIV infection are devastating because the CD$_4$ T-lymphocyte plays a critical role in the human immune response. It is responsible directly or indirectly for induction of a wide array of lymphoid and non-lymphoid cell functions. These effects include activation of macrophages, induction of functions of cytotoxic T cells, Natural killer cells, B cells and secretion of variety of soluble factors that induce growth and differentiation of lymphoid cells and affect haematopoietic cells (Geo, 1998).

Monocytes and macrophages play a major role in the dissemination and pathogenesis of HIV infection. Certain subsets of monocytes express the CD$_4$ surface antigen and therefore bind to the envelope of HIV. The HIV co-receptor on monocyte and macrophage is the CCR5 chemokine receptor.

Infected pulmonary alveolar macrophages may play a role in the interstitial pneumonitis seen in certain patients with AIDS.

Macrophage tropic strains of HIV predominate early after infection, and these strains are responsible for initial infections even when the transmitting source contains both M-tropic and T-tropic viruses.

It's believed that monocytes and macrophages serve as major reservoirs for HIV in the body. Unlike the CD$_4$ T cell, the monocyte is relatively refractory to the cytopathic effects of HIV, so that the virus can not only survive in this cell but can be transported to various organs in the body such as lungs and brain (Volk et al., 1996).

Lymphoid organs play a central role in HIV infection. Lymphocytes in the peripheral blood represent only about 2% of the total lymphocyte pool, the
remainder being located chiefly in lymphoid organs where specific immune responses are generated.

Throughout the course of untreated infection, even during the stage of clinical latency, HIV is actively replicating in lymphoid tissues where it is ideal for the establishment and spread of HIV infection. Cytokines are released activating a large pool of CD4 T cells that are highly susceptible to HIV infection. As the late stage of HIV disease progresses, the architecture of the lymphnodes becomes disrupted.

Neureologic abnormalities are common in AIDS and occur to varying degrees in 40-90% of patients. These include HIV encephalopathy, peripheral neuropathies and most serious AIDS dementia complex. Both direct and indirect pathogenic mechanisms might explain the neuropsychiatric manifestations of HIV.

Virus may enter the brain through infected monocyte and release cytokines that are toxic to neurons as well as chemotactic factors that lead to infiltration of the brain with inflammatory cells. HIV has been found in a limited number of neurons, oligodendrocytes and astrocytes.

HIV infected persons develop both humoral and cell mediated response against HIV related antigens. Antibodies to a number of viral antigens develop soon after infection, but the response pattern against specific viral antigens changes over time as patient progress to AIDS.

Antibodies to envelope glycoproteins (gp 41, gp 120, gp 160) are maintained but those directed against the core protein (p24) decline. The decline of Anti p24 may herald the beginning of clinical signs and other immunologic markers of progression.

Most infected individuals make neutralizing antibodies against HIV. The neutralizing antibodies can be measured in vitro by inhibiting HIV infection of susceptible lymphocyte cell lines.
Cytotoxic T- lymphocytes recognize env, pol and gag genes products, this reactivity is mediated by major histocompatibility complex restricted to CD₃ and CD₈ lymphocytes. The env specific reactivity occurs in nearly all infected people and decreases with progression of disease. Natural killer cell activity has also been detected against HIV-1 gp 120. It's not known which of these host responses is important in providing protection against HIV infection. A problem confronting AIDS vaccine research is that the correlates of the protective immunity are not known, including the relative importance of humoural and cell-mediated immune response (Geo et al., 1998).

2.5. Transmission:

HIV is transmitted during sexual contact (including genital-oral sex), through parenteral exposure to a contaminated blood or blood products and from mother to child acquiring infection through breast-feeding, during birth and during prenatal period is probable (Volk et al., 1996). The presence of other sexually transmitted diseases such as syphilis, gonorrhea, or chancroid increases the risk of sexual HIV transmission as much as a hundred folds, because the inflammation and sores facilitate transferring of HIV across mucosal barriers. Since the first description of AIDS, promiscuous homosexual activity has been recognized as a major risk factor for acquisition of the disease.

Furthermore health care workers usually get infection by HIV following a needle stick with contaminated blood.

There has been considerable concern that in rare circumstances other types of transmission may occur such as through "casual" contact with HIV infected persons or insect vectors, but there is no evidence of virus transmission under these casual conditions (Volk et al., 1996).
2.6. Peripheral Blood in AIDS:

Cytopenias are commonly seen in association with HIV infection, anemia, thrombocytopenia, neutropenia, lymphocytopenia, monocytopenia, or combinations of any or all of these can occur in over 90% of patients with AIDS. The microenvironment of the marrow may also be altered by HIV infection of stromal cells including fibroblasts, endothelial cells, reticular cells, macrophages, osteoclasts, and steatocytes, resulting in dysregulation of hematopoietic cell growth with reduced hematopoiesis. (Moses et al., 1998) Anemia is present in over half of patients early in the course of AIDS and in nearly all AIDS patients late in the course. The anemia is often normochromic and normocytic, typical of anemia of chronic disease, and iron stores are increased by measurement of serum ferritin. Though CD$_{34}$ stem cells poorly express CD$_{4}$ receptors and, hence, are relatively resistant to HIV infection, mononuclear cells are infected and produce cytokines such as TGF, TNF, and IL-1 that suppress hematopoiesis. Cytopenias can be potentiated by drug therapy including zidovudine (ZDV), ganciclovir, amphotericin B, or trimethoprim-sulfamethoxazole and may require dose reduction or cessation of therapy. Though a positive direct antiglobulin test may be present in up to 43% of HIV-infected persons, hemolytic anemia is uncommon. (Volberding et al., 2003) anemias in AIDS occasionally may results from use of drugs that act as folate antagonists (trimethoprim-sulfamethoxazole).

Thrombocytopenia is commonly seen in about a third of AIDS cases, is rarely severe, and may result from peripheral consumption (splenomegaly, immune complexes) or from decreased marrow platelet production. Thrombocytopenia may also appear in some HIV-infected persons prior to development of clinical AIDS, and most cases are due to platelet consumption.

Lymphopenia is present in about a third of AIDS cases due to the decrease in T4 lymphocytes.
Neutrophilia may indicate bacterial sepsis. Bone marrow failure leading to death in patients with AIDS is very uncommon. (Jacobson et al., 1997; Moses et al., 1998; Volberding et al., 2003; Meynard et al., 1997).

Neutropenia that accompanies HIV infection can increase the risk for infection or worse the course of infection. Neutropenia can be common in patients with AIDS and is a risk factor for both bacterial and fungal infections. The most common cause for neutropenia is drug therapy. Neutropenia can result from involvement of bone marrow by opportunistic infections, from pharmacologic therapies such as trimethoprim-sulfamethoxazole, and from direct effects of HIV through accelerated apoptosis. Both chemotaxis and phagocytic functions of neutrophils also appear to be impaired.

Thrombosis is more likely to occur when the CD₄ count is less than 200/µl. (Volberding et al., 2003; Copur et al., 2002). The antiphospholipid syndrome has been reported in association with HIV infection (Leder et al., 2001).

**2.7. Acquired immunodeficiency syndrome (AIDS):**

AIDS is a group of clinical syndrome caused by HIV, characterized by profound immune suppression with diverse clinical features, including opportunistic infection, malignancies and central nervous system. (Osmond et al., 1991) Acute renal failure remains common among hospitalized patients with HIV and is associated with chronic kidney disease, liver disease, and increased mortality (Christina, et al. 2006).

**Classification**

Two major classification systems currently are in use: the U.S. Centers for Disease Control and Prevention (CDC) classification system (CDC, 1992) and the World Health Organization (WHO) Clinical Staging and Disease Classification System. (WHO, 2007)
The CDC classification of HIV disease was first put forth as a categorization of HIV related symptoms into four groups and was explicitly for "public health purposes" and not "intended as a staging system," although it was frequently treated as if it were a staging system in the AIDS literature (CDC, 1986). Staging is disease classification that aims primarily to make groupings that have different prognosis and can be used in guiding treatment decisions. Stages attempt to classify disease in a progressive sequence from least to most severe, each higher stage having a poorer prognosis or different medical management than the preceding stage. The current CDC classification system from the revision in 1993 combines three categories of the CD4 count with three symptom categories and are closer to a staging system. This description of its intended use is close to the use of a staging system (CDC, 1993).

**CD4 T-lymphocyte categories:**

- **Category 1:** > 500 cells/µl (or CD4% > 28%)
- **Category 2:** 200-499 cells/µl (or CD4% 14% - 28%)
- **Category 3:** < 200 cells/µl (or CD4% < 14%)

**Categories of clinical conditions:**

**Category A**

A symptomatic HIV infection, persistent generalized lymphadenopathy acute (primary) HIV infection with accompanying illness or history of acute HIV infection in an adolescent (>13 years) with documented HIV infections. Conditions listed in categories B and C have not occurred.

**Category B**

Consists of symptomatic conditions in an HIV infected adolescent or adult that are not included among conditions listed in clinical Category C and that meet at least one of the following criteria:
A\ The conditions are attributed to HIV infection or are indicative of a defect in cell mediated immunity.
B\ the conditions are considered by physicians to have a clinical course or to require management that is complicated by HIV infection.

**Category C**
Includes the clinical conditions listed in the 1993 AIDS surveillance case definition. For classification purposes, once a Category C condition has occurred, the person will remain in Category C (Dennis, 1998).
The WHO system classifies HIV disease on the basis of clinical manifestations that can be recognized and treated by clinicians in diverse settings, including resource-constrained settings, and by clinicians with varying levels of HIV expertise and training. (WHO. 2007)

S: When a patient presents with a diagnosis of HIV infection, review the patient's history to elicit and document any HIV-related illnesses or symptoms

O: Perform a complete physical examination and appropriate laboratory studies

A: Confirm HIV infection and perform staging.

P: Evaluate symptoms, history, physical examination results, and laboratory results, and make a staging classification according to the CDC or WHO criteria(WHO. 2007).

2.8. **Clinical manifestation**:
The clinical manifestations of HIV disease include five stages, primary infection, and early, middle-, advanced-, and late-stage HIV disease (Osmond Dennis H 1998). These stages of HIV infection goes on the primary infection, dissemination of virus to lymphoid organ, clinical latency, elevated HIV
expression, clinical disease, and death. There is much individual variation in the clinical manifestations of individual patients in the same disease stage and in the rate of progression through the stages.

The duration between primary infection and progression to clinical disease in untreated individuals averages about 10 years (Bacchetti & Moss, 1989). The estimate varies with the age at which infection occurs and is significantly shorter in infants and in older adults and varies even between infection at age 20 and infection at age 40. Death usually occurs within 2 years after the onset of clinical symptoms (Rosenberge et al., 1994).

2.8.1. Primary infection:

Often symptoms less, but when present appear to result from immunopathic, lymphocytopathic, and neuropathic. Sometimes symptoms present with an infection mononucleosis-like illness, during 2 weeks to 3 months after exposure. Reported manifestations include fevers, fatigue, malaise, arthralgias, myalgias, lymphadenopathy, splenomegaly, anorexia, nausea and vomiting, diarrhea, pharyngitis, headache and retroorbital pain, meningitis and encephalitis, neuropathy, myelopathy, maculopapular rash, and mucocutaneous ulceration. Lymphadenopathy onset typically occurs in the second week of the syndrome and may be generalized or preferentially affect the occipital, axillary, and cervical groups, fevers, rash, sore throat, night sweats, malaise, Lymphadenopathy, diarrhea, and relative and absolute lymphocytosis in the peripheral blood. There may be mouth and genital ulcers, a profound transient decrease in CD4 lymphocyte. (Schacker et al., 1998; Keet et al., 1993; Schechter et al., 1990; Pantaleo et al., 1997; Niu et al., 1993; Henrard et al., 1997).

2.8.2. Early and Middle stage:

The early and middle stage of HIV disease followed the primary infection after at least three-month. In which the level of detectable virus in peripheral blood
drop dramatically and antibodies rise (Phillips, 1996). This period of clinical latency may last for as long as 10 years, during which the patients may show, depressed count of CD4 lymphocyte (from normal to 200 to 300 cells/µl). Through the subsequent early and middle stages of HIV disease, the level of virus detectable in the peripheral blood often remains low (Pantaleo et al., 1998).

Clinical manifestations are usually minimal or no symptoms, other individuals, however have subjective systemic symptoms, such as fatigue and a few have fevers. Many patients have painless stable lymphadenopathy, which in some cases actually regresses as HIV disease advances. Allergy to skin testing becomes increasingly probable as the CD4 count falls beyond 400 cells/µl, with obvious implications for tuberculosis screening (Pantaleo et al., 1998).

2.8.3. Advanced HIV disease:

Untreated patients with manifestation of advanced HIV diseases typically have CD4 counts below 200 cells/µl, increased plasma HIV RNA levels, and clinical manifestation indicative of sever immunocompromise, and in addition opportunistic infections commonly occur. In the absence of prophylaxis, *Pneumocystis Carinii Pneumonia* (PCP), is the most common such infection. In-patients with HIV dementia complex, manifestations typically become profound. Systemic symptoms including fever, fatigue, and weight loss are prominent, often without identifiable secondary causes. *Cryptosporidium* diarrhea is a common problem with limited treatment options, and the probability that it will resolve or remit decrease HIV disease progress. HIV plasma RNA levels typically are high (Ioachim, 1990).

2.8.4. Late-stage HIV disease:

As the disease advances further and the CD4 count drops below 50 cells/mm, additional opportunistic infection as well as central nervous system occur commonly, and in homosexual males, existing *Kaposi Sarcoma* may become
extensive, and cause disfigurement and clinically significant edema. Central nervous system toxoplasmosis, *Cryptococcal Meningitis*, *cytomegalovirus* (CMV) disease, and *Mycobacterium Avium Complex* (MAC) disease is frequent. Infection such as MAC, CMV, *Strongyloides Stercoralis*, *Herpes Zoster* or *Tuberculosis*, which normally colonize superficially or are limited to an organ system or a local anatomic region, may invade tissue or disseminate widely. Simultaneous clinically significant infection by more than one pathogen is common. Secondary symptoms become increasingly problematic, including anorexia, nausea, vomiting, diarrhea, mal-absorption, muscle wasting, and weakness.

Death eventually results from extensive disease of vital organs, most commonly the lungs and presumably from effects of circulating toxins, electrolyte abnormalities hematopoietic and circulatory failure and autonomic nervous system damages (Nguyen *et al.*, 1995).

Receiving an AIDS diagnosis does not necessarily mean that the diagnosed person will die soon; some people have lived for many years after their diagnosis. This is even more the case today with the availability of highly active antiretroviral therapy (HAART), which has helped extend the lives of thousands of people living with HIV and AIDS. In addition, many opportunistic infections can be prevented or treated successfully. This has substantially increased the longevity and quality of life of people living with HIV/AIDS. (San Francisco AIDS Foundation. 2008).

### 2.9. Laboratory diagnosis of HIV:

Techniques used in the detection of HIV include: screening tests, supplemental tests, detection of viral antigen, viral RNA/DNA and direct isolation of the virus. (Sangeeta Joshi. 2008). Also other measurements for monitoring
disease progression in HIV-1 infected individuals, such as determination of the CD$_4$ are helpful.

**2.9.1. ELISA:**

Standard screening tests for HIV include detection of anti-HIV antibodies in blood by enzyme immunoassays (EIA/ELISA). These tests use virus antigens (proteins) to detect the circulating antibodies. The bound antibodies are detected by a colorimetric reaction. These tests become positive 3-12 weeks after infection and have more than 99% sensitivity and greater than 95% specificity. The tests can detect infection with HIV-1 and HIV-2. However, false positives may occur in people with multiple blood transfusions, malignancies, alcoholic hepatitis etc. False negatives can occur very early in the infection or in patients who do not produce enough detectable antibodies or very late in the infection (Sangeeta Joshi. 2008).

**2.9.2. Rapid tests:**

These are tests that can yield results in < 30 min. The results are read by naked eye. When performed correctly, these are accurate and have wide utility in a number of testing situations (Dr Sangeeta Joshi. 2008). Application includes emergency room, physician's office, autopsy room, and smaller blood banks.

**Types**

1. Dot blot assays/ tridot
2. Particle agglutination . Also latex agglutination can be used for initial screening; CD$_4$/CD$_8$ ratio correlates infection of HIV (How et a., 1998).
3. HIV spot and comb tests
4. Fluorimetric microparticle technologies
   One class of rapid tests is the 'dot blot' or 'immunoblot'; they produce a well-
circumscribed dot on the solid phase surface if the test is positive. There is a
color change because of antigen-antibody reaction.

Disadvantages:

1. Subjective interpretation
2. Difficult to read if the laboratorian is color blind.

Advantages:

1. Rapid HIV assays have proven particularly useful for testing pregnant women
   in labor who have not received prenatal care.
2. It is helpful in detecting HIV-2 infection which can not be detected by ELISA.
   (Archana Sharma, YS Marfatia.2008)

2.9.3. Western Blot (WB):

It is a more specific assay (Archana Sharma, YS Marfatia.2008); In this antibodies
against numerous proteins are detected.
Env (gp160, gp120, and gp41)
gag (p55, p24, and p17)
pol (p66, p51, and p31)
(Archana Sharma, YS Marfatia.2008)

The western blot method is based on the electrophoresis separation of major
proteins of an infectious agent in a two- dimensional agarose gel matrix. A
suspension of the organism against which antibodies are being produced is
mechanically or chemically disturbed and the solubilized antigen suspension is placed at an end of a polyacrylamide (polymer) gel. Under influence of electrical current, the proteins migrate through the gel. Most bacteria or viruses contain several major proteins that can be recognized based on their position in the gel after electrophoresis. Smaller proteins travel faster and migrate further in the lanes of the gel than do the larger proteins. The protein bands are transferred from the gel to a nitrocellulose or other type of thin membrane and the membrane is treated to immobilize the proteins. The membrane is then cut into many thin strips, each carrying the pattern of protein bands. When patient serum is layered over the strip, antibodies present can be used to determine whether the patients are infected with the agent or not. Antibodies against microbes with numerous cross-reacting antibodies, such as T. pallidum, B. burgdorferi, herpes simplex virus types 1 and 2 and HIV, are identified more specifically using this technology than by methods that test for only one general antibody type (Volk et al., 1996).

2.9.4. Qualitative PCR:

PCR is a method that amplifies viral nucleic acid to allow for its detection in patient specimens. It is a particularly specific and sensitive test which can pick up very small numbers of viral particles (M Fearon.2005). PCR is very useful in the diagnosis of HIV infection in babies born to infected mothers. Babies will carry maternal antibody up to approximately 15 months of age and, therefore, the antibody test is not a reliable indicator of infection in these children.

Because of the very specific nature of the primers used in the HIV-1 DNA PCR assay, caution should be used in assessing a negative result in an infant if the mother is likely to have HIV-2 or a non-B HIV infection. It may be prudent to ensure that the HIV DNA PCR assay is effective in detecting the mother's HIV
infection if there is any likelihood that the case is not an HIV subtype B infection. In North America, this would be a very uncommon finding.

PCR may also be useful in resolving indeterminate Western blot results and testing immunocompromised individuals who may not mount an antibody response.

2.9.5. Quantitative RNA PCR and genotyping:

Quantitative RNA PCR must only be used to monitor HIV-positive individuals before or during antiretroviral therapy (Zhang M et al 2002). It is used in conjunction with CD4 counts and general clinical assessments to ascertain when therapy should be started. It is also used to help determine the patient's response to therapy. Genotyping is used to monitor the development or presence of drug resistance in patients before or during therapy. It is also used to assist physicians in their choice of antiretroviral drug combinations for the patient (Zhang M et al 2002), (Hirsch MS, et al 2003).

Quantitative PCR should not be used as a diagnostic test for HIV because false positives and false negatives can occur in these circumstances.

2.10. T.Lymphocytes:

CD4 and CD8 molecules are T cell surface glycoproteins that are expressed on mutually exclusive subsets of mature T cells with distinct patterns of MHC restriction (Howcroft et al., 1993). CD4 and CD8 serve as accessory molecules by facilitating interaction of T cells with APCs or CTL target cells respectively.

The function of CD4 and CD8 are intricately associated with TCR function and therefore accessory molecules are often called coreceptoers. Approximately 65% per cent of peripheral α B- positive T cells express CD4, and 35 % express CD8 (Howcroft et al., 1993).
2.10.1. CD4 cells:

CD4 cells are the major targets for HIV. Also known as helper T-cells, are a type of lymphocyte, which is a white blood cell that plays an important role in the immune system. Lymphocytes control the body's ability to recognize and fight infections and cancers. CD4 lymphocytes help to identify, attack, and destroy specific bacteria, fungi, and other germs that infect the body. In addition, they regulate the production of antibodies, and cytokines. CD4 cells are produced in bone marrow and differentiated in thymus, then migrates to spleen and lymph nodes, they circulate throughout the body in the bloodstream. HIV binds to the surface of CD4 cells, enters them, and either reproduces immediately, killing them in the process, or remains in a resting state, reproducing when the cell becomes active (Lang et al., 1989).

The number of CD4 cells in a blood sample, which is called CD4 count, ranges from 500 to 1600 cells/µl. The CD4 cell count is a laboratory marker of the strength of the immune system. CD4 T lymphocyte subset count is one of the best measurements for monitoring disease progression in HIV infected individuals (De Wolf,. 1988 Stein,. 1992).

The plasma CD4 lymphocyte count (CD4 count, T-helper cell count) has been used since the beginning of the HIV epidemic to indicate disease stage, although it has some limitations (Hoover et al., 1992; Malone et al., 1990). Individual determinations may vary significantly. Identifiable causes of variation include time of day, short-term changes in health, and experience of the clinical laboratory performing the test. Nevertheless, over many months, the decline in CD4 cells reflects progression of untreated HIV disease, and prior to 1996, used CD4 counts to indicate disease stage. The CD4 count alone does not always reflect the clinical status of an HIV-infected individual; untreated individuals with similar CD4 counts may have very different functional status, frequency of opportunistic
infections, and constitutional symptoms and signs. Further, the CD4 cell numbers reflect only one aspect of immunocompetence. Treatment with potent antiretroviral therapy often results in return of CD4 counts to high levels that probably mask other immune system defects, thus somewhat complicating staging of disease. To understand a patient's disease stage in the era of potent antiretroviral therapy, the clinician should consider both the most recent CD4 count and the patient's lowest past count ("nadir CD4 count"). Even in patients who have responded to antiretroviral therapy with markedly increased CD4 counts, however, subsequent decline of over time indicates continuing progression.

2.10.2. Function of CD4:

Two important functions in the activation of CD4 cells:

1- CD4 serve as cell-cell adhesion molecules, by virtue of it is specific affinity for class 11 MHC molecules. The invariant CD4 molecule bind via it is two N-terminal Ig-like domains to non polymorphic B2 domain of the class 11 MHC molecule.

2- CD4 molecule may transduce signals or facilitate TCR complex mediated signal transduction upon binding class 11 MHC molecule there by promoting the subsequent functional responses of class restricted T cells.

2.10.3. Function of CD8:

The CD8 molecule is thought to have the same two general roles as CD4 namely in cell to cell adhesion and signal transudation. First CD8 serve as cell to cell adhesion molecules by binding to nonpolymorpige immunoglobulin-like α3 domain of class 1 MHC molecule there by stabilizing the interaction of class 1 MHC-restricted T cell (usually a CTL) with a target cell bearing class 1 MHC-associated antigen. Second the CD8 molecules may tranduce signal or may facilitate TCR: CD3-mediated signal transduction upon binding class 1 MHC
molecules, thereby promoting subsequent function responses of class 1-restricted T cells.

2.11. Methods for CD<sub>4</sub> & CD<sub>8</sub> lymphocyte enumeration:

Accurate and reliable measures of CD<sub>4</sub> T lymphocytes are essential for the assessment of the immune system of HIV-infected persons (Turner <i>et al.</i>, 1994; Hoover <i>et al.</i>, 1992). The pathogenesis of AIDS is largely attributable to the decrease in T lymphocytes that bear the CD<sub>4</sub> receptors (DeWolf <i>et al.</i>, 1988; Smith, 1990). Progressive depletion of CD<sub>4</sub> T lymphocytes is associated with an increased likelihood of clinical complication (Hanson <i>et al.</i>, 1995; Stein <i>et al.</i>, 1992). Consequently, the Public Health Service (PHS) has recommended that CD<sub>4</sub> T lymphocyte levels be monitored every 3-6 months in all HIV-infected persons (CDC, 1992). The measurement of CD<sub>4</sub> T cells levels has been used to establish decision points for initiating pneumocystis carinii pneumonia prophylaxis (CDC, 1995) and antiretroviral therapy (National Institutes of Health, 1990) and to monitor the efficacy of treatment (Goldman <i>et al.</i>, 1996- De Gruttola <i>et al.</i>, 1994). CD<sub>4</sub> T lymphocyte levels are also used as prognostic indicators in patients who have HIV disease (Fahey <i>et al.</i>, 1990; CDC, 1994) and recently have been included as one of the criteria for initiating prophylaxis for several opportunistic infections that are sequel of HIV infection (CDC, 1995; CDC, 1993). Moreover, CD<sub>4</sub> T lymphocytes levels are a criterion for categorizing HIV-related clinical conditions by CDC’s classification system for HIV infection and surveillance case definition for AIDS among adults and adolescents (CDC, 1992).

Other observations made in the era prior to the advent of combination therapy also suggest it is likely that nearly all HIV-infected persons will eventually lose CD4 lymphocytes and progress to AIDS in the absence of effective treatment. For example, in about 10 years of follow-up of 288 men HIV-seropositive at baseline in the San Francisco General Hospital Study (1983-1993), only one
(0.3%) maintained a CD4 lymphocyte count above 700/µl throughout follow-up and nearly all showed some worsening of other laboratory values predictive of AIDS, such as anti-p24 antibody levels, B-2 microglobulin, or neopterin. Most HIV-positive persons, even with near-normal CD4 lymphocyte counts, show functional lymphocyte abnormalities that suggest their long-term immune functioning will be impaired. There are no clearly documented instances of persons who appear to have cleared a longstanding HIV infection, although there is a report of transient infection in an infant (Berdberg & Raden et al., 1995).

CD4T lymphocytes can be counted by different techniques either automated or manually.

2.12. Treatment and control:

Numbers of different antiretroviral agent have been developed. Of these, the RT inhibitor AZT has been the most widely used. Major toxicities of AZT include neutropenia, anaemia and myopathy. Several other nucleotide analogues that act as chain termination in the synthesis of the DNA provirus. Such as DDI didanosine, Zalcitabine (ddc) and stavudine (d4T), but they toxicities. Non competitive inhibitor of the RT includes nevirapine and delavirdine. In general, all TR inhibitors are limited by their low potency, their side effects and the development of resistance. Protease inhibitors such as saquinavir have the advantage of being able to inhibit HIV replication in cells that are chronically infected (Butera & Folks, 1992). AZT delays the development of opportunistic infection and prolongs survival. How early to start AZT remains controversial. Most clinical experts however, agree on recommending AZT when the CD4 cell count drops below 500. The development of clinically significant AZT resistance, the development of opportunistic infection, and progressive weight loss and constitutional symptoms, seem to comprise a good indication for need to change treatment. In USA,
recommendations have been issued for the use of AZT in HIV infected pregnant women to reduce prenatal transmission. Currently there is no effective vaccine available and limited clinical trials are conducted to test candidate vaccines (CDC, 1994b).

Immune reconstitution could be found in part of HIV/AIDS patients after receiving highly active anti-retroviral therapy (HAART). Its influencing factors are very complex. Many factors, such as the demographic factor, stage of HIV disease, pattern of therapy, sort of co-infections, baseline of CD4; T-cell count, abnormal immune activation, related immune phenotype, and virologic factors, all could influence the immune reconstitution (Wang et al., 2009).

3. Visceral Leishmaniasis in Sudan:

VL has been reported in Sudan since the beginning of 20th century (Zijlstra & Elhassan, 2001). The first case reported in Sudan was described by Neave in 1904. The patient was a boy aged 8-9 years from Bahr-El Gazal and diagnosed at Omdurman Teaching Hospital. The patient was suffering from diarrhoea and splenomegaly and that was the second case reported in Africa after the first case which was reported in Tunis 1903 by Laveran. The second case reported in Sudan was an Egyptian soldier who had contracted the disease in Singa town and died in Cairo in 1907 (Cummins, 1908). As a result of a continuous reporting of cases of VL, two governmental commissions were set in the period between 1908-1911 to investigate the disease in the Eastern and the Blue Nile provinces. One commission was headed by (Bousfield 1908-1910) a pathologist of the Welcome Research Laboratory. The other one was by two pathologists, (Archibal and Marshal 1911-1913).

The commission was closed in October 1913 and it was concluded that VL was endemic in the Eastern province particularly Mafaza, Singa, Kassala, Galabat,
Gadarif, Sofi and Tomat (Satti, 1958). It was reported that dogs had been the naturally infected reservoir (Marchal, 1911).

Since then, researchers continued investigating different aspects of VL between 1931 and 1959, which revealed valuable information in a series of papers under the heading: "Studies in Leishmaniasis in Anglo-Egyptian Sudan". They studied different aspects of the disease involving vector, transmission and clinical presentation as well as treatment (Henderson, 1937; Kirk & Satti, 1940a; Kirk & Lewis, 1940, Kirk & Satti, 1947).

VL occurs in sporadic small outbreaks and sometimes attaining an epidemic form in the Upper Nile province and South Fung District of the Bule Nile province (Stephanson, 1940; Satti, 1958, 1963a). A major epidemic took place in 1988 among displaced southerners from Bentiu, where a lot of people died (El Hassan et al., 1993). The dynamics presented in (Figure 1) suggest that Southern Sudan is currently between epidemics and provide a warning that cases may rise dramatically in coming years. In 2006, a total of 1,117 cases were reported, 65.4% of which were primary cases; the remainder were either relapses or cases of post-kala-azar dermal leishmaniasis (Jan H. Kolaczinski et al., 2008). The WHO estimated that at least 400,000 people were killed by the disease and it had been well known by natives in Sudan by different name e.g. Seimaih, Dobal, Elsaid fever (Thomson, 1911).
In Sudan, the disease was found to be endemic in the Eastern Region: Kassala, Gadarif, Hawata, Rahad and Dinder River up to River Atbara (El Safi et al., 2002; Zijalstra & El Hassan, 2001). In the center it encroaches on the Blue Nile State and to the south in the Upper Nile province and Equatoria State (Kapoeta). Different foci were detected in Darfur, near the Nuba Mountains (Kirk & Lewis, 1955).

The disease had been reported in Khartoum province by (Shamsedin 1969) and later by (Kadaru 1995), who reported two cases from El Alafon who had never left their home. Small outbreaks were reported in El- Khogalab village where it affected 22 school children's (Hamza et al., 1976) and Eidumish, in the White Nile (El Safi, 1996) as well as Wad zaki near Eldweim in the White Nile State (Kadaru, 1995). The disease attained seasonal variations, with preponderance between October and February (Henderson, 1937; Kirk, 1939). There was increased incidence of transmission during the rainy season which was supposed to be due to
humidity and low temperature (Manson & Bahar., 1987). It most commonly affects young adults and was common among children in Bentiu in the South. (Henderson, 1937) Males are more affected than females (Kirk, 1939). It is general a disease of low socio-economic classes where malnutrition acts as a provoking factor (Kirk, 1959; Yousif & WHO, 2001).

For a considerable time, workers in the field of leishmaniasis in the Sudan investigated the possible reservoir hosts (Kirk, 1956; Satti, 1958; Hoogostraal et al., 1963). They observed that small outbreaks had occurred among military personnel who had been stationed in remote uninhabited lands. In 1963 Hoogostraal and his group demonstrated natural *Leishmania* infection in the grass rats Arvican niloticus luctuosus, the rodent, Rattus Rattus and Accomys species which co-incited with the distribution of *P. orientalis* in Malakal. Accordingly, they concluded that nile grass rats were the possible reservoir hosts of VL.

Sixel (1987) found massive *Leishmania* infection in a Jakal, which was suggested as a possible reservoir in southern Sudan. Recently a preliminary report in two villages (Bandigues & Um Salala), in Eastern Sudan by (Mukhtar, et al 2000) described the detection of *L. donvani* antibodies by DAT and ELISA in animal's sera such as sheep, cows, dogs, camels and donkeys. In regular episemiological studies, which were carried out by (Elsafi, et al., 1997-2000) in Barbar el Fugara, a village in Gedarif State (eastern Sudan), they found the parasite in lymph node culture of dogs, rodents and donkeys.

In Sudan the pioneer work of Kirk and his colleagues on the vector showed that *P. orientalis* was the main vector of VL in Sudan (Kirk & Lewis, 1947). It is widely distributed in the endemic area of VL, and is found in Kassala, Blue Nile, Darfur, Melut, Malakal and Kapoeta (Kirk & Lewis, 1955). *P. orientalis* was found to be naturally infected with *leishmania* (El Naiem, 1996). The flies rest during the day in the cavities of trees in wet seasons and in cracks in the soil during dry

PKDL is most commonly reported in India (WHO, 1996). It is known to occur in the Sudan during or shortly following treatment (Kirk and Satti, 1940 and Satti 1963a). It can even occur without past history of VL (EL-Hassan et al., 1992) or as puncture rash (Kirk & Satti., 1940) or as nodular depigmented areas (Kirk & Macdonalds, 1940). It affects most commonly the face, limbs, extensor surfaces of the arm and trunk.

4. HIV/AIDS in Sudan:

Years of civil war and limited epidemiological data make it difficult to generalize about the status of HIV/AIDS in the Sudan. It is generally agreed that the country is in the early stages of a generalized HIV/AIDS epidemic, with an almost exclusively heterosexual transmission pattern, and indications of higher infection rates in the South than in the North. Internally displaced persons, refugees, prison inmates and members of the armed forces are some of the groups believed to be particularly vulnerable to HIV infection. In addition, high risk groups such as commercial sex workers are known to exist in the country, and studies to further understand their risk profile are planned. From a regional perspective, the country is very important to the treatment scale up efforts in the WHO Eastern Mediterranean Region (EMRO), because 73% of people requiring ART in the region have been estimated to reside in Sudan (WHO, 2006). The first case of AIDS was reported in 1986, and by December 2006, the adult national prevalence was 1.6%, indicating that Sudan is now in generalized epidemic (http://www.unaids.org/en/HIV_data/epi2006/default.asp) (Jorge Alvar. 2008)

The most reliable available indication of the extent of the epidemic is the 2002 Situation Analysis study conducted in the government-controlled parts of the
country (11 out of 16 states in the north and 3 in the south). The study yielded HIV prevalence rates ranging from 0.5% for soldiers, 1% for antenatal care attendees, truck drivers, and Internally Displaced Persons (IDPs), 2.5% among female tea sellers, to 4.4 % among female sex workers. More recently, results of limited sentinel surveillance testing conducted during 2004 by SNAP yielded prevalence rates of 0.95% (18/1900) among pregnant women, 1.9% (9/465) among symptomatic STD patients, and 2.3% (33/1436) among TB patients.

It needs to be clarified that there have been two prominently cited estimates of the HIV sero-prevalence for the general population (1.6% as well as 2.6%), in various documents discussing HIV/AIDS in Sudan. However the 1.6% figure appears to have been derived from aggregation of the overall HIV positive rate among all the samples from the various groups tested during the epidemiological component of the 2002 Situation Analysis study (SNAP, 2002). But the methodology employed by the study would suggest that it was designed to determine sero-prevalence for the various groups studied, rather than the “general population prevalence” from the overall positive rate among the samples collected. The estimate of 2.6 % was based on a UNAIDS/ WHO modeling for the whole country (2003).

The limited available data from Southern Sudan suggest relatively higher infection rates, compared to the north. Studies have yielded general population prevalence rates ranging from 2.7 in Yei (2003) to 7% in Yambio (2000). In the city of Juba, (located in the south but administered by the government), 10% of female tea sellers (a group some of whose members are believed to engage in casual / commercial sex), were found to be HIV positive in 2002. These higher rates of infection would suggest that IDPs hailing from the affected areas may have higher HIV rates than their host communities, this being made even more likely by the disruptions to family cohesion and sexuality norms that being an IDP could
cause. Unfortunately this assumption could also reinforce a perception of relative safety and inaction for communities in the North of Sudan. It therefore worth pointing out that despite citation of the report of one survey yielding of a 5% HIV positive rate among IDPs in Khartoum, this level of infection is not supported by other data. For instance, the 2002 situation analysis study yielded an IDP HIV prevalence of 1%, the same as that obtained for pregnant women in all the states, and results of sentinel testing among IDP antenatal mothers in Khartoum, done by SNAP and CARE during 2004, yielded a positive rate of 1.6% (11/700), very similar to the 1.5% (5/400) for general population pregnant women in the Red Sea state, for the same period (SNAP, 2004).

HIV/AIDS prevalence, based on scarce epidemiological and behavioral information, is estimated by UNAIDS to be around 2.3% in the adult population. Rates of HIV infection have been estimated by Sudan National AIDS Control Programme (SNAP) to be at 1.6% nationwide. For Southern Sudan, estimates vary from 1% to 7.2% with alarming rates among certain population (SNAP, 2006).

5. Overview of Leishmania/HIV co-infection:

Leishmania/HIV co-infection is emerging as an extremely serious, new disease and it is increasingly frequent (WHO 2000). There are important clinical, diagnostic, chemotherapeutic, epidemiological and economic implications of this trend. Although people are often bitten by sandflies infected with Leishmania protozoa, most do not develop the disease. However, among persons who are immunosuppressed (e.g. as a result of advanced HIV infections, immunosuppressive treatment for organ transplants, haematological malignancy, auto-immune diseases), cases quickly evolve to a full clinical presentation of severe leishmaniasis.

AIDS and VL are locked in a vicious circle of mutual reinforcement. On the one hand, VL quickly accelerates the onset of AIDS (with opportunistic diseases
such as tuberculosis or pneumonia) and shortens the life expectancy of HIV-infected people. On the other hand, HIV spurs the spread of VL. AIDS increases the risk of VL by 100-1000 times in endemic areas. This duo of diseases produces cumulative deficiency of the immune response since Leishmania parasites and HIV destroy the same cells, exponentially increasing disease severity and consequences. The HIV/AIDS pandemic has modified the natural history of leishmaniasis. HIV infection increases the risk of developing VL by 100 to 2,320 times in areas of endemicity, reduces the likelihood of a therapeutic response, and greatly increases the probability of relapse. At the same time, VL promotes the clinical progression of HIV disease and the development of AIDS-defining conditions. Both diseases exert a synergistic detrimental effect on the cellular immune response because they target similar immune cells (Jorge Alvar et al., 2008).

Patients who are coinfected with visceral leishmaniasis and HIV could be a reservoir for development and spread of drug-resistant strains (Van Griansven, 2010). VL is considered a major contributor to a fatal outcome in co-infected patients. Lately, however, use of tri therapy, where it is available, has improved the Prognosis for Leishmania/HIV cases. Leishmaniasis can be transmitted directly person to person through the sharing of needles, as is often the case among intravenous drug users. This group is the main population at risk for co-infection.

Although cases of co-infection have so far been reported in 33 countries worldwide, most of the cases have been notified in south western Europe (Desjeux, 2001) 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 (Desjeux & UNAIDS, 1998).
Leishmania/HIV co-infections impose specific difficulties in terms of diagnosis and treatment. The usual clinical features (fever, weight loss, liver and spleen enlargement, inflammation of the lymph nodes) are not always present. The clinical diagnosis can also be made difficult by associated diseases such as cryptosporidium, disseminated cryptococcosis, cytomegalovirus infection or mycobacterial infection.

The serological diagnosis is falsely negative in 42.6% of co-infected patients. HIV-positive patients have difficulty in producing antibodies against new infectious agents, especially at a late stage or during relapses. Consequently, there is a need to use two or more serological tests and antigens freshly prepared in the laboratory to increase sensitivity.

Although multiple localizations are frequent (blood, skin, digestive tract, lungs, central nervous system), parasitological diagnosis can be difficult and has to be repeated to orient the treatment. Bone marrow aspirate (BMA) remains the safest and most sensitive technique, but spleen aspirate and liver biopsy are also used. When BMA cannot be performed, the search for Leishmania can be conducted in peripheral blood samples.

Treatment for co-infected patients is aimed at clinical and parasitological cures and prevention of relapses. Unfortunately, in such patient treatment failure, relapses due to drug resistance and drug toxicity are very common. In south-western Europe, follow-up studies using pentavalent antimonials, the same first-line drug used to treat classic leishmaniasis, show a positive response in 83% of cases. However, 52% of patients relapse within a period of
one month to three years, with the number of relapses ranging from one to four. The main alternative drugs include pentamidine, amphotericin B and amphotericin B encapsulated in liposomes. This encapsulation reduces the occurrence of side-effects, but relapses still occur and the drug remains extremely expensive.

Leishmania/HIV co-infections can lead to epidemiological changes which modify the traditional patterns of zoonotic VL. Co-infected patients harbour a high number of Leishmania in their blood so there is also a risk of them becoming reservoirs of the disease (that is, infective for the sandfly vector) as in anthroponotic foci in Bangladesh, India, Nepal and East Africa. Consequently, there is an increased risk of future epidemics.

Experimentally, sandflies can be infected through a blood meal containing a very small quantity of blood from co-infected patients. The quantity may be less than the content of a needle. As 71.1% of co-infected patients in south-western Europe are intravenous drug users, transmission of Leishmania has occurred through the sharing of syringes in this population group.

5.1. Global Leishmania/HIV co-infection:

Cases of Leishmania /HIV co-infections are being reported more frequently in various parts of the world. The first case of leishmaniasis associated with HIV infection was reported in 1985. (Jorge Alvar et al. 2008) It is anticipated that the number of Leishmania /HIV co-infections will continue to rise in the coming years and there are indications that cases are no longer restricted to endemic areas.

The overlapping geographical distribution of VL and AIDS is increasing due to
two main factors: the spread of the AIDS pandemic in suburban and rural areas of the world, and the simultaneous spread of VL from rural to suburban area.

*Leishmania*/HIV co-infections are considered a real threat, especially in south-western Europe. Of the first 1 700 cases of co-infection which have been reported to the World Health Organization (WHO) from 33 countries worldwide up to 1998, 1 440 cases were from the region: Spain (835); Italy (229); France (259); and Portugal (117). Of 965 cases retrospectively analyzed, 83.2% were males, 85.7% were young adults (20-40 years old) and 71.1% were intravenous drugs users.

Most co-infections in the Americas are reported in Brazil, where the incidence of AIDS has risen from 0.8 cases per 100 000 inhabitants in 1986 to 10.5 cases per 100 000 inhabitants in 1997. As HIV transmission has spread into rural areas, VL has simultaneously become more urbanized--especially in north-eastern Brazil--increasing the risk of overlapping infection.

### 5.2. Leishmania /HIV Co-infection in Africa:

The number of cases of *Leishmania*/HIV co-infection is expected to rise in Africa owing to the simultaneous spread of the two infectious diseases and their increasingly overlapping geographical distribution, complicated by mass migration, displacement, civil unrest, and war.

In general, the reported cases of *Leishmania*/HIV co-infection in Africa are a very modest estimation and would substantially increase if active surveillance were implemented throughout the continent. Ethiopia has a well-organized system of detection, management and reporting of co-infection. Kenya and Sudan began surveillance in 1998 and Morocco has also established a surveillance centre.

In East Africa, cases of *Leishmania*/HIV co-infections have been reported in Djibouti (10), Ethiopia (74), Kenya (15), Malawi (1) and Sudan (3). West Africa
has no official surveillance system yet, but several cases have been reported: Cameroon (1), Guinea Bissau (1), Mali (4) and Senegal (2). In North Africa, cases have been reported in Algeria (20) and Morocco (4).

5.3. 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 (Elsafi et al 1998-1999).

Up to 1998, only three cases of coinfection had been identified. Since then, the reported prevalence in hospital-based studies was 5% (3/60) in Khartoum between 1998 and 1999, 9.4% (5/53) in Khartoum in 2002, and 8.1% (3/37) and 3.6% (3/84), respectively, in 2002 and 2003 in Gedaref state. Because of the spread of both VL and HIV, an increase in the prevalence of coinfection is expected. Most coinfected cases present with typical manifestations of VL (WHO, 2007).

In Sudan 1990-1998 only 3 cases of leishmania and HIV co-infection were reported (Poredes et al 1997). The numbers are expected to rise owing to factors such as increasing mass migration, displacement, civil unrest, war and sex work.

Elsafi et al (1997) 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 (MSF-Holland Report, 1996-1999) and HIV infection (SNAP Report 1997-1998).

Generally HIV co-infected VL cases presented with clinical features typical of VL although splenomegaly was found only in 75% of the cases (Elsafi et al 1998-1999).
In clinicoepidemiological and serological studies of Leishmania/HIV Co-infection in Sudan, which carried out by (Elsafi et al 1999-2005) to determine the prevalence of HIV infection among patients with VL and to investigate the clinical, epidemiological and serological features of coinfected cases in the Sudan, three series of confirmed VL cases (total 150 patients were studied, 5%(3/60), 9.4(5/53) and 8.1(3/37) of the VL in the three studied series, respectively, were positive for HIV. In addition, HIV infection was confirmed in 5 serologically confirmed VL cases bringing the total of co-infected cases to 15. Two other co-infected patients (one parasitologically and one serologically confirmed) had been detected prior to the study period. The 17 co-infected cases included 14 males and 3 females. Their age ranged rom 15 to 51 years; 9 were married and 8 were single. Three of them presented with relapse and the rest had newly acquired VL. They included 4 farmers, 4 labourers, one soldier, one student, two house wifes, one merchant and the others were civil servants. They belonged to different tribes including Nuba, Nuier, Barno, Tama, Masalit, Hawsa, Dago and Nubian. Most of the cases came from eastern and southern Sudan, others came from western, central and northern parts of the country and two cases came from Ethiopia. The results of risk factors for HIV infection in the second series showed that two cases reported illegal sexual contact, one had blood transfusion, and one had STD. None of them was intravenous drug user and all of them were resident in or had a history of travel to a VL endemic area. All the cases complained of fever and fatigue. Other symptoms included loss of weight (88%), abdominal pain (71%), couh (59%), anorexia (59%), diarhoea (35%) and epistaxis (29%). The clinical signs included pallor (82%), splenomegaly (76%), hepatomegaly (65%), lymhpadenopathy (59%), chest signs (35%), oral candidiasis (29%), skin lesions (24%) and jaundice (18%). Laboratory result showed that all the o-infected patients were anemic; three of them had hypochromic microcytic anemia. The TWBCs count was< 2000 in two
cases, between 2000-5000 in two cases and >5000 in one case. A comparison of series of clinical and epidemiological variables between HIV+ve (5) and HIV –ve (48) confirmed VL cases showed that the absence of splenomegaly (P= 0.002), lymphadenopathy (P= 0.024), the presence of epistaxis (P= 0.018), and abdominal pain (P= 0.018) were significantly more observed in co-infected cases. However, no significant difference relating to possible risk factors was observed between the two groups.

In addition, the performance of serological tests (IFAT, ELISA, WB) and Katex in the diagnosis of VL in 12 parasitologically and/ or serologically (DAT) confirmed patients co-infected with HIV was assessed. Parasitological confirmation was obtained in 50% (6/12) of the cases. The DAT was positive in 67%, IFAT in 91% and both the ELISA and WB were positive in all cases. With the exception of one smear negative patient, the Katex was positive in all (7) the cases; the test was strongly positive in 5 patients.
Justification & Objectives

1.6. Justification:

VL is a major health problem and has been reported in Sudan since the beginning of 20th century (Zijlstra & Elhassan, 2001).

The first case of HIV infection in Sudan was reported in 1987 in a hemophilic boy. Since then infection increased and AIDS become a serious health problem (Hashim et al., 1998)

An emerging health problem is the increasing occurrence of VL/HIV co-infection. In view of the fact that both VL and HIV infections are increasing in the Sudan, VL cases co-infected with HIV may be found. Leishmaniasis acts also as an endemic disease which has a high mortality and morbidity rate in Sudan.

As the disease is a reticulo-endotheliosis and the parasite sequestrates in many visceral organs such as bone marrow, liver, spleen and lymph nodes so, the infiltration of these organs may affect their functions. Since these organs are the most vital, loss of function leads to other serious complications, so the study of biochemical parameters may be considered indicator for stage of disease severity and guide for intervention.

1.7. Objectives:

General Objective:
- To confirm the prevalence of Leishmania/HIV co-infection in Sudan.

Specific Objectives:
- To measure renal and liver profiles among Sudanese VL/HIV co-infected patients.
- To correlate CD4 cells count with renal and liver profiles.
II. Materials & Methods

2.1: Study area:

The present study was conducted at Tabarak-Allah Rural Hospital in Gedarif state, Eastern Sudan; the area is recognized as highly endemic for VL (Dereure et al., 2003). Tabarak-Allah village is located about 100 Km from Gedarif town; it is surrounded by other villages including Barbar, Birkat Nowrin and Mashroua Firsan from which VL suspects are referred. The hospital is specialized in Kala-azar diagnosis and treatment and is under the responsibility of the Ministry of Health, Gedarif State. It has a laboratory equipped with one light Microscope (Olympus CH2), Refrigerator, Centrifuge, Water bath, Geimsa stains and all necessary glassware. The laboratory is capable of carrying out important routine investigations for most of the important endemic diseases such as malaria, typhoid, leishmaniasis and tuberculosis. The laboratory staff consists of one technician, two laboratory assistants and a laboratory attendant. The other hospital staff includes one doctor, two medical assistants, nurses, midwives and a nutritionist.

The other site used for the recruitment of cases in this study was Al-Azaza Centre, Sinnar State, an area which is also recognized as endemic for VL (EL Hassan et al., 1995). It is located about 200 Km from Aldindir town and is surrounded by other villages including Nor Algalil and Um bagara from which VL suspects are referred. The Medical center is specialized in Kala-azar diagnosis and treatment and is under the responsibility of the Ministry of Health, Sinnar State. It has a laboratory equipped with one light Microscope (Olympus CH2), Refrigerator, Centrifuge, Water bath, Geimsa stains and all necessary glassware. The laboratory is capable of carrying out important routine investigations for most of the important endemic diseases such as malaria, typhoid, leishmaniasis and tuberculosis. Its laboratory staff they are one technician, one laboratory assistant
and a laboratory attendant. The other staff includes a medical officer, two medical assistants and nurses.

2.2 Study subjects

The study subjects included VL cases and VL cases that are found to be co-infected with HIV in addition to confirmed HIV seropositive individuals.

a. VL cases:

VL clinically suspects who attended Tabarak-Allah Rural Hospital and Al-azaza Health Center between April 2008-April 2010 were recruited for the study. The criteria for clinical selection were based on the presence of fever for two weeks or more, splenomegaly and/or lymphadenopathy after the exclusion of malaria.

b. HIV positive individuals

All HIV seropositive individuals who had been referred to VCT Centre (Gedarif Teaching Hospital) between March and April, 2010 were enrolled in this study.

c. VL/ HIV co infected cases

This group was identified by HIV testing of all confirmed (parasitologically and/or serologically) VL cases. All confirmed VL cases that were found to be positive for HIV were included in this study group.

2.3 Study Design

This study is a descriptive study. An eligible clinical suspect was duly informed about the objectives and procedures of the study. In case he/she agrees to participate, a consent form is signed, before the patient is enrolled in the study.

In addition, a cross sectional study was done to determine the biochemical parameters in known seropositive HIV individuals.
2.4 Sample size

A total of 99 VL confirmed cases were enrolled in the present study. In addition 30 HIV seropositive individuals were recruited from Al Gadarif Teaching Hospital.

2.5 Ethical considerations

The consent of VL cases was obtained for all the study subjects. They were invited to be included in the study and informed about the objectives and procedures of the study. In case he/she agreed to participate, an informed consent form (ICF) was signed before the subject was enrolled in the study.

2.6 Recruitment of study subjects

A coded enrollment number was given for each enrolled patient. The personal, epidemiological and clinical data of each VL suspect were collected using a standard form. Each patient was examined by the clinician in charge and the clinical information was recorded in the Clinical Report Form (CRF).

2.7 Collection of Samples

From each enrolled VL suspect, the following samples were collected: lymph node and/or bone marrow aspirates, venous blood and urine, for parasitological, serological, biochemical and immunological tests respectively. Each blood sample was divided into two parts: one part was collected in EDTA anti coagulant containers for molecular study; the rest of sample was drawn in plain containers, left over night at R.T and centrifuged at 3200 rpm for three minutes. Sera were collected in 1.5 ml eppindorf tubes and frozen at (-20c) for performance of DAT and HIV screening. Urine samples were collected from all VL suspects in sterile containers at the time of diagnosis for the detection of leishmania urinary antigen.
2.8 Parasitological study

A. Lymph node aspiration:

The diagnosis of VL was confirmed by the demonstration of amastigotes in Geimsa stained smear of lymph node aspirate of VL suspects. Specimens (n/157) from inguinal lymph node of VL suspects were collected. Slides were cleaned with gauze and labeled with patient name and laboratory 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. A 21 G sterile needle was inserted in the center of the gland at a right angle to the skin, and the gland was squeezed with the left hand while the needle rotated. The fluid then came up through the needle, needle was rapidly drawn. The piston of the syringe was drawn back and the needle containing lymph node aspirate was attached. After the fluid was discharged on the slide it was quickly mixed, left to dry, fixed with methanol and stained with Geimsa. It was then examined for amastigotes under the microscope using X100 oil immersion (Evans, 1989; WHO, 1996).

B. Bone marrow:

A bone marrow aspiration was carried out by the medical officer in charge. The patient was asked to lie on his or her abdomen (prone position) or on his/her side (lateral decubitus position) Bain BJ et al (2005). The skin is cleaned, and a local anesthetic such as lidocaine was injected to numb the area. Patients may also be pretreated with analgesics and/or anti-anxiety medications, although this is not a routine practice.

Typically, the aspirate was performed first. An aspirate needle was inserted through the skin until it abuts the bone. Then, with a twisting motion, the needle was advanced through the bony cortex (the hard outer layer of the bone) and into
the marrow cavity. Once the needle was in the marrow cavity, a syringe was attached and used to aspirate ("suck out") liquid bone marrow. A twisting motion was performed during the aspiration to avoid excess content of blood in the sample, which might be the case if an excessively large sample from one single point is taken.

Subsequently, the biopsy is performed if indicated. A different, larger trephine needle was inserted and anchored in the bony cortex. The needle was then advanced with a twisting motion and rotated to obtain a solid piece of bone marrow. This piece was then removed along with the needle. The entire procedure, once preparation was completed, typically takes 10–15 minutes.

If several samples were taken, the needle was removed between the samples to avoid blood coagulation (Bain BJ et al 2005).

2.9 Serological Tests

The DAT was performed for the demonstration of specific anti *leishmania* antibodies. In addition, the urine latex agglutination test for the detection of *Leishmania* antigen was carried out. Furthermore, the rK39 strip test was carried out for the detection of specific anti leishmania antibodies in serum.

2.9.1 Direct agglutination Test

A. Performance of DAT

All the collected sera from the parasitologically confirmed or Suspects VL patients were tested with the DAT as described by Harith et al,( 1995).

Two fold serial dilution of serum in 0.9 % (wt/vol) NaCl with 2%( wt/vol) gelatin and 80% (wt/vol)2ME and urea(0.02%wt/vol) were added to Leishmania antigen using micro titration plates with 96 V shape wells. A DAT titer of 1:≥ 3200 was reported as a positive result (Harith et al., 1995).
**B. Reading DAT plates**

The plate was placed on a white background and the titer was estimated compared to a negative control. The titer was the highest dilution that showed visible clumping and aggregation in comparison to the negative control (plate). The cut of 1:3200 titer seems to be positive.

**2.9.2 Latex Agglutination Test (KATEX)**

A Katex Kit (Kalon Biological, UK) was performed according to Attar, et al (2001); the Kit contains Latex beads that have been coated with IgG anti leishmania antibodies.

**A. Principle**

The principle of test is based on agglutination of Latex beads that have been coated with IgG in presence of leishmania urinary antigen.

**B. Performance**

The test was performed according to Attar et al (2001.) Sufficient sample tubes were labeled for each patient to be tested, 250µl to 1000µl of urine sample were transferred into sample tubes, then put in a rack, immersed in boiling water and heated for 5 minutes. It was, allowed to cool to ambient temperature before applying the test.

All reagents were brought to room temperature and the bottle of latex was shaken well before testing. 50µl of the treated (boiled) urine sample were placed in the reaction zone on the glass slide, and then one drop of the well mixed latex was added. Both were mixed until they were completely homogeneous and covering the whole surface of the reaction zone. The glass slide was rotated and rocked consistently for 2 minutes in both clockwise and anti-clockwise directions to ensure complete mixing. Then the degree of agglutination was recorded under a good stream of daylight. A positive and negative control was included in each patch.
C. Interpretation of results

Agglutination was interpreted using the cross system as follow:

++++ Majority of the latex agglutinates and moves to the reaction area.

+++ Agglutination resembles chalk dust scattering onto surface.

++ Clear agglutinated particles were seen against the background of latex.

+ Agglutination was just visible as compared to the negative control.

− No visible agglutination.

All the first three grades of agglutination were regarded as positive.

2.9.3 rK 39 strips

The test strip membrane (InBios, Seattle, WA, USA) was coated on the bottom with a band of rK39 antigen, and on the top with immobilized anti-protein-A antibody to detect IgG. A protein A-gold conjugate was used as the immunochromatographic detection reagent. One drop of serum was placed on the absorbent pad at the bottom of the strip, three drops of the test buffer were added to the pad, and the mixture was allowed to migrate up the strip by capillary action. After 10 min, the appearance of a red upper (control) line indicated the presence of IgG and proper test functioning; a red lower line indicated the presence of anti-rK39 IgG and a positive test result.

This rK39 test was performed using LEISMANIA Strip Quick Test (CYPRESS DIAGNOSTICS).

2.10 HIV Testing

a- VL cases

Sera of all parasitologically/or serologically confirmed VL patients were screened for HIV by ELISA (Microlisa HIV). All reactive samples were confirmed using another kit (Bio Rad kits) at the National Health Laboratory.

Microwell ELISA is a highly sensitive enzyme immunoassay for the detection of antibodies and antigens to HIV-1(including subgroup o&c) and HIV-2 in human
serum or plasma. The test was performed in accordance with the manufactures instructions.

Briefly, all controls and samples were diluted in the ratio of 1:4 using conjugate 1, and incubated at 37°C for 60 minutes. The wells were washed five times with working wash solution and were reacted with 100µl of conjugate 2 for 30 minutes at 25°C. After a final wash as described previously, then 80µl of working substrate was added to each well and incubated at R.t for 30min in dark. Then 100µl of stop solution was added. After incubation for 2 minutes at room temperature, then the absorbance was measured at 450nm in Elisa reader.

Negative and positive serum control samples were included for each patch. The cutoff value was calculated as follow: 
Cut off value=NCX+0.2.

Test specimens with absorbance value greater than or equal to the cut off value were reactive by Microlisa-HIV and vice versa.

b- HIV seropositive individuals

The diagnosis of HIV infection had been confirmed in all the HIV seropositive individuals who were included in the present study from the VCT centers. The laboratory diagnosis for those individuals was performed at VCT in the hospital and included the following: screening with rapid test and ELISA in addition to confirmation by another kit of third generation of ELISA.

2.11 Biochemical Studies

Renal and liver profile was done for 99 VL confirmed patients and 30 HIV seropositive individuals.

2.11.1 Liver profile

a. Serum bilirubin (Total & Direct) measurement

Was measured by colorimetry using corning 252 colorimeter and making of the fact that absorbance is proportional to the concentration of the analyte in the
sample. We add one ml of sulphanilic acid(Total) to the tube of the test total and also 1ml of sulphanilic acid(Direct) to the tube of the test direct then add 50 µl of sodium nitrite to each tube of test then we add 100 µl of serum to each tube, incubate for 5 minutes at room temperature, and read the color developed at 520 nm using the colorimeter. For indirect bilirubin we subtract direct from total to get it. (Jandarasik and Groff method).

b. Serum albumin measurement

We add 10 µl sample to one ml of bromocresol green reagent, incubate for 10 minutes at 37°C which converted to green colored compound that measured colorometrically at 580 nm.(BCG method).

c. Serum Total protein measurement

We add 25µl of sample to 2ml of biuret reagent, after incubation for 10 minutes at room temperature colored compound will be formed which is measured colorometrically at 540 nm.(Biuret method).

d. Serum Aminotransferases (ALT, AST) activity measurement

Sample was added to substrate, reaction occurs and the rate of reaction is monitored kinetically to get the enzyme activity at 340 nm using spectrophotometer.

e. Serum Alkaline phosphatase activity measurement

Sample was added to substrate, reaction occurs and the rate of reaction is monitored kinetically to get the enzyme activity at 340 nm using spectrophotometer.

2.11.2 Renal profile

a. Blood urea and serum creatinine determination

For urea 10µl of serum was added to one ml urease containing reagent, after incubation at 37°C we add one ml of hypochlorite solution, incubate for 10 minutes
at 37C then read the color at 580nm using colorimeter.(Berthlots enzymatic method).

For creatinine we remove proteins by adding 0.5 ml of DW ,0.5ml 10% sodium tungestate, 0.5 ml 2/3N sulphuric acid and 0.5 ml of serum, centrifuge for 3 minutes then react the supernatant with alkaline picare to form color measured colormeterically at 520 nm.(Jaff chemical method).

b. Serum Electrolytes (Na+, K+),Ca+2 determination

We add 10 µl of sample to one ml orthocresolphthalen reagent, after incubation at 37C for 5 minutes Calcium will react with the reagent to form color compound that measured colormeterically at 580.(Cresolphthalien method).

0.2 ml of serum/plasma was taken and added to 19.8 ml of deionized water, the diluted sample was aspirated with the flame photometer, Na+ ,K+ burned with propane gas are excited and gain energy then they loss this energy in a form of light which measured by photometric mode.(Flame emission photometer).

c. Serum Uric acid determination

We add 25 µl of serum to one ml of uricase containing reagent, incubate for 10 minutes at 37C then read the color at 520 nm by photometer which is directly proportional to the concentration.(Uricase enzymatic Method).

2.12. Statistical Methods and Data Analysis

The analysis of the result was done manually, using statistical T.Test, chi-square and correlation tests. The test was considered as significant when the P. value < 0.05. Further, the data was processed and formulated into tables and figures using the Microsoft Excel Computer Program.
III. RESULTS

3.1. VL patients:

3.1.1. Epidemiological, clinical and diagnostic results:

During this study 157 suspects with VL were admitted to Tabarak Allah kalazar center in Gadarif State (137 suspects) and Al-azaza center in Sinnar State (20 suspects). 99 patients were confirmed parasitologically and serologically. There were 57 (57.6%) males and 42 (42.4%) females, their ages ranged from 2-75 years (mean of ages was 16 years), 75 patients (75.8%) were less than 20 years old and 24 patients (24.2%) were more than 20 years old. All patients presented with a history of fever, usually for two weeks or more, splenomegaly and lymphadenopathy. Other symptoms included abdominal distention (65%), weight loss (85%), anorexia (55%), epistaxis (45%), diarrhea (40%), and cough (60%), (Table: 1). 35 (35.8%) persons were masalit, 5 (5.4%) Tama, 2 (2.3%) Dago, 10 (10.9%) Zabarma, 1 (1.1%) Tera, 2 (2.2%) Salhab, 5 (5.4%) Gemir, 1 (1.1%) Bani mora, 4 (4.3%) Bani amir, 4 (4.3%) Foor, 9 (9.8%) Hawsa, 1 (1.1%) Ethiopian, 5 (5.4%) Bargo, 1 (1.1%) Aranga, 2 (2.2%) Maratia, 3 (3.3%) Falata and 2 (2.2%) Noba, (Table: 2).

3.1.2. Renal and liver profiles:-

Of the 99 confirmed VL cases, jaundice was detected in Nine (9.1%), elevated AST activity in 85 (85.9%), elevated ALT 19 (19.2%), elevated ALP in 55 (55.6%). Low albumin level in 78 (78.8%), hyperprotienamia in 40 (40.4%), Azotamia in 7 (7.3%), hypocalcaemia in 48 (48.5%), hyponatraemia in 89 (89.9%), hypokalaemia in 29 (29.3%) and hyperkalaemia in 11 (11.1%) (Table: 3). Two (2.4%) patients had renal failure.
(Table .1): Clinical Features of parasitologically and serologically confirmed VL patients.

<table>
<thead>
<tr>
<th>Symptoms/Signs</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>100 %</td>
</tr>
<tr>
<td>Loss of Weight</td>
<td>85%</td>
</tr>
<tr>
<td>Abdominal Distention</td>
<td>65 %</td>
</tr>
<tr>
<td>Cough</td>
<td>65 %</td>
</tr>
<tr>
<td>Anorexia</td>
<td>55%</td>
</tr>
<tr>
<td>Epistaxis</td>
<td>45 %</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>40 %</td>
</tr>
<tr>
<td>Vomiting</td>
<td>15%</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>100 %</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>45 %</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td>100 %</td>
</tr>
<tr>
<td>Pallor</td>
<td>80 %</td>
</tr>
<tr>
<td>Jaundice</td>
<td>9.1%</td>
</tr>
</tbody>
</table>

(Table .2): Tribe Distribution of parasitologically and serologically confirmed VL patients.

<table>
<thead>
<tr>
<th>Tribe</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Masalit</td>
<td>35(38%)</td>
</tr>
<tr>
<td>Tama</td>
<td>5 (5.4%)</td>
</tr>
<tr>
<td>Dago</td>
<td>2 (2.2%)</td>
</tr>
<tr>
<td>Zabarma</td>
<td>10 (10.9%)</td>
</tr>
<tr>
<td>Tera</td>
<td>1 (1.1%)</td>
</tr>
<tr>
<td>Salhab</td>
<td>2 (2.2%)</td>
</tr>
<tr>
<td>Gemir</td>
<td>5(5.4%)</td>
</tr>
<tr>
<td>Bani mora</td>
<td>1(1.1%)</td>
</tr>
<tr>
<td>Parameter</td>
<td>Number of patients</td>
</tr>
<tr>
<td>----------------------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>Bani Amir</td>
<td>4 (4.3%)</td>
</tr>
<tr>
<td>Foor</td>
<td>4 (4.3%)</td>
</tr>
<tr>
<td>Hawsa</td>
<td>9 (9.8%)</td>
</tr>
<tr>
<td>Ethiopian</td>
<td>1 (1.1%)</td>
</tr>
<tr>
<td>Bargo</td>
<td>5 (5.4%)</td>
</tr>
<tr>
<td>Aranga</td>
<td>1 (1.1%)</td>
</tr>
<tr>
<td>Maratia</td>
<td>2 (2.2%)</td>
</tr>
<tr>
<td>Falata</td>
<td>3 (3.3%)</td>
</tr>
<tr>
<td>Noba</td>
<td>2 (2.2%)</td>
</tr>
</tbody>
</table>

(Table 3): Renal and liver profiles of parasitologically and serologically confirmed VL patients.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin more than 1.0 mg/dl</td>
<td>9 (9.1%)</td>
</tr>
<tr>
<td>AST more than 42 U/L</td>
<td>85 (85.9%)</td>
</tr>
<tr>
<td>ALT more than 42 U/L</td>
<td>19 (129.3%)</td>
</tr>
<tr>
<td>ALP more than 113U/L</td>
<td>55 (55.6%)</td>
</tr>
<tr>
<td>Total protein more than 8.1 g/dl</td>
<td>40 (40.4%)</td>
</tr>
<tr>
<td>Total protein Less than 6.0 g/dl</td>
<td>2 (2%)</td>
</tr>
<tr>
<td>Albumin Less than 3.5 g/dl</td>
<td>78 (78.8%)</td>
</tr>
<tr>
<td>Sodium less than 135 mmol/l</td>
<td>89 (89.9%)</td>
</tr>
<tr>
<td>Potassium less than 3.5 mmol/l</td>
<td>29 (29.3%)</td>
</tr>
<tr>
<td>Potassium More than 5.1 mmol/l</td>
<td>11 (11.1%)</td>
</tr>
<tr>
<td>Calcium less than 8.0 mg/dl</td>
<td>48 (48.5%)</td>
</tr>
<tr>
<td>Urea more than 50 mg/dl</td>
<td>9 (9.1%)</td>
</tr>
</tbody>
</table>
Fig(1): Frequency of age in VL Patients

- 1 --- 10
- 11 --- 20
- 21 - 30
- 31 - 40
- < 40
Figure 2: Sex distribution of confirmed VL patients

![Sex distribution graph]

Fig: 3 Frequency distribution of T.Bilirubin level in confirmed VL Patients

![Frequency distribution graph]

Normal Values: (Up to 1 mg/dl)
Fig: 4 Frequency distribution of AST level in confirmed VL Patients

Normal Values: (Up to 40 IU/L)

Fig: 5 Frequency distribution of ALT level in confirmed VL Patients

Normal Values: (Up to 40 IU/L)
Fig: 6 Frequency distribution of ALP level in confirmed VL Patients

Normal Values: (Up to 113 IU/L)

Fig: 7 Frequency distribution of T.Protien level in confirmed VL Patients

Normal Values: (6.0 - 8.0 g/dl)
**Fig: 8 Frequency distribution of Albumin level in confirmed VL Patients**

Normal Values: (3.5 - 5.0 g/dl)

**Fig: 9 Frequency distribution of Urea level in confirmed VL Patients**

Normal Values: (10 - 50 mg/dl)
Fig: 10 Frequency distribution of Creatinine level in confirmed VL Patients

Normal Values: (0.4 - 1.4 mg/dl)

Fig: 11 Frequency distribution of Calcium level in confirmed VL Patients

Normal Values: (8.1 - 10.4 mg/dl)
Fig: 12 Frequency distribution of Sodium level in confirmed VL Patients

Normal Values: (135 - 150 mmol/l)

Fig: 13 Frequency distribution of Potassium level in confirmed VL Patients

Normal Values: (3.5 - 5.5 mmol/l)
3.2. VL/HIV co-infected patients:

3.2.1. Epidemiological, clinical and diagnostic results:

Seven (7.1%) out of 99 parasitologically and serologically confirmed VL cases were co-infected with HIV. Their age ranged from 13-26 years (mean 22 years); the prevalence of HIV among adult VL patients (age of more than 16 years) was 16.7% (6/36), (figure: 14), (figure: 15). All patients showed fever, splenomegaly and lymphadenopathy. One (14.3%) patient was Masalit, 1(14.3%) Tama, 2(28.6%) Dago, 1(14.3%) Zabarma, 1(14.3%) Hawsa and 1(14.3%) was Noba.

3.2.2. Renal and liver profiles of HIV/VL co-infected patients:

One (14.3%) out of 7 HIV/VL co-infected cases had elevated ALT, 7(100%) elevated AST, 6(85.7%) elevated ALP activity, 2(28.6%) hyperprotienamia, 5(71.4%) hypoalbuminaemia, 3(42.9%) hypocalcaemia, 7(100%) hyponatraemia, 2(28.6%) hypokalamia and 1(14.3%) hyperkalaemia (Table: 4).

(Table .4): Renal and liver profiles of HIV/VL co-infected patients.

<table>
<thead>
<tr>
<th>Pt No.</th>
<th>Sex</th>
<th>Age /Year</th>
<th>T.BIL</th>
<th>D.BIL</th>
<th>ALT</th>
<th>AST</th>
<th>ALP</th>
<th>T.P</th>
<th>ALB</th>
<th>UREA</th>
<th>CREAT</th>
<th>CA</th>
<th>Na</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>25</td>
<td>0.8</td>
<td>0.19</td>
<td>20</td>
<td>79</td>
<td>129</td>
<td>8.51</td>
<td>2.6</td>
<td>18</td>
<td>0.7</td>
<td>8.1</td>
<td>120</td>
<td>3.5</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>18</td>
<td>0.8</td>
<td>0.34</td>
<td>34</td>
<td>290</td>
<td>145</td>
<td>8.09</td>
<td>3.7</td>
<td>30</td>
<td>0.6</td>
<td>9.2</td>
<td>126</td>
<td>3.4</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>24</td>
<td>0.8</td>
<td>0.26</td>
<td>31</td>
<td>112</td>
<td>192</td>
<td>7.25</td>
<td>3.5</td>
<td>25</td>
<td>0.6</td>
<td>7.4</td>
<td>130</td>
<td>5.2</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>13</td>
<td>0.4</td>
<td>0.21</td>
<td>29</td>
<td>98</td>
<td>127</td>
<td>6.22</td>
<td>3.3</td>
<td>25</td>
<td>0.6</td>
<td>7</td>
<td>114</td>
<td>4.1</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>17</td>
<td>0.5</td>
<td>0.24</td>
<td>156</td>
<td>230</td>
<td>110</td>
<td>6.81</td>
<td>3.1</td>
<td>39</td>
<td>1.2</td>
<td>7.9</td>
<td>114</td>
<td>3.9</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>18</td>
<td>0.6</td>
<td>0.25</td>
<td>13</td>
<td>67</td>
<td>260</td>
<td>7.37</td>
<td>3.3</td>
<td>26</td>
<td>0.7</td>
<td>8.3</td>
<td>116</td>
<td>3.7</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>26</td>
<td>0.5</td>
<td>0.15</td>
<td>21</td>
<td>90</td>
<td>137</td>
<td>8.03</td>
<td>3.6</td>
<td>22</td>
<td>0.6</td>
<td>8.7</td>
<td>130</td>
<td>3.9</td>
</tr>
</tbody>
</table>
Fig: 14 Prevalence of HIV among VL patients.

Fig: 15 Prevalence of HIV among adults VL patients.
3.3. HIV seropositive individuals:

3.3.1. Epidemiological, clinical and diagnostic results:

A total of 30 previously diagnosed HIV seropositive individual’s who had been referred to VCT Centre at AlGadarif Teaching Hospital for ART were included in this study. 23 (76.7%) of them were males, and 7 (23.3%) were females. Their age ranged between 30 and 54 years (mean of age was 40 years). Three (10%) out of them had DAT significant titer for VL, and 3(10%) had border line titer. 6(20%) were Masalit, 1(3.3%) Tama, 1(3.3%) Zabarma, 1(3.3%) Gemir, 1(3.3%) Bani Mora, 4(13.3%) Bani Amir, 2(6.7%) Foor, 3(10%) Ethiopian, 1(3.3%) Bargo, 1(3.3%) Falata, 2(6.7%) Noba, 1(3.3%) Araki, 1(3.3%) Kawahla, 1(3.3%) Habania, 1(3.3%) Dianka, 1(3.3%)Gawamaa, 1(3.3%) Sholok and 1(3.3%) Gaali. (Table: 5).

3.3.2. Renal and liver profiles:-

Three (10%) out of the 30 studied HIV positive individuals were jaundiced, 12(40%) with elevated ALT, 11(36.7%) with elevated AST, 11(36.7%) with elevated ALP, 6(20%) with hyperprotiemaemia, 3(10%) with hypoprotienamia, 19(63.3%) with hypoalbuminaemia, 3(10%) with Azotamia, 16(60%) with hyponatraemia, 2(6.7%) with hyperkalaemia, 10(33.3%) with hypokalamia, 13(43.3%) with hyperuricaemia, 1(3.3%) with hypouricaemia, 16(53.3%) with hypocalcaemia and one (3.3%) had renal failure (Table:6).

3.3.3. CD4:

CD4 T.cells counts were done by VCT center personnel for 30 HIV seropositive individuals during ART using flowcytometry. 18(60%) of the cases had CD4 count < 200 cell/ µl, 10(33.3%) had CD4 between 200- 500 cell/ µl, and 2(6.7%) had CD4 > 500 cell/ µl (Table: 7).
(Table 5): Tribe Distribution of HIV seropositive Individuals.

<table>
<thead>
<tr>
<th>Tribe</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Masalit</td>
<td>6 (20%)</td>
</tr>
<tr>
<td>Tama</td>
<td>1 (3.3%)</td>
</tr>
<tr>
<td>Zabarma</td>
<td>1 (3.3%)</td>
</tr>
<tr>
<td>Gemir</td>
<td>1 (3.3%)</td>
</tr>
<tr>
<td>Bani mora</td>
<td>1 (3.3%)</td>
</tr>
<tr>
<td>Bani Amir</td>
<td>4 (13.3%)</td>
</tr>
<tr>
<td>Foor</td>
<td>2 (6.7%)</td>
</tr>
<tr>
<td>Ethiopian</td>
<td>3 (10%)</td>
</tr>
<tr>
<td>Falata</td>
<td>1 (3.3%)</td>
</tr>
<tr>
<td>Bargo</td>
<td>1 (3.3%)</td>
</tr>
<tr>
<td>Noba</td>
<td>2 (6.7%)</td>
</tr>
<tr>
<td>Araki</td>
<td>1 (3.3%)</td>
</tr>
<tr>
<td>Kawahla</td>
<td>1 (3.3%)</td>
</tr>
<tr>
<td>Habania</td>
<td>1 (3.3%)</td>
</tr>
<tr>
<td>Dainka</td>
<td>1 (3.3%)</td>
</tr>
<tr>
<td>Gawamaa</td>
<td>1 (3.3%)</td>
</tr>
<tr>
<td>Sholok</td>
<td>1 (3.3%)</td>
</tr>
<tr>
<td>Gaali</td>
<td>1 (3.3%)</td>
</tr>
</tbody>
</table>
(Table .6): Renal and liver profiles of HIV seropositive individuals.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin more than 1.0 mg/dl</td>
<td>3 (10%)</td>
</tr>
<tr>
<td>AST more than 42 U/L</td>
<td>11 (36.7%)</td>
</tr>
<tr>
<td>ALT more than 42 U/L</td>
<td>12 (40%)</td>
</tr>
<tr>
<td>ALP more than 113U/L</td>
<td>11 (36.7%)</td>
</tr>
<tr>
<td>Total protein more than 8.1 g/dl</td>
<td>6 (20%)</td>
</tr>
<tr>
<td>Total protein Less than 6.0 g/dl</td>
<td>3 (10%)</td>
</tr>
<tr>
<td>Albumin Less than 3.5 g/dl</td>
<td>19 (63.3%)</td>
</tr>
<tr>
<td>Sodium less than 135 mmol/l</td>
<td>18 (60%)</td>
</tr>
<tr>
<td>Potassium less than 3.5 mmol/l</td>
<td>10 (33.3%)</td>
</tr>
<tr>
<td>Potassium more than 5.1 mmol/l</td>
<td>2 (6.7%)</td>
</tr>
<tr>
<td>Uric Acid more than 7.1 mg/dl</td>
<td>13 (43.3%)</td>
</tr>
<tr>
<td>Uric Acid less than 2.0 mg/dl</td>
<td>1 (3.3%)</td>
</tr>
<tr>
<td>Calcium less than 8.0 mg/dl</td>
<td>16 (53.3%)</td>
</tr>
<tr>
<td>Urea more than 50 mg/dl</td>
<td>4 (13.3%)</td>
</tr>
</tbody>
</table>

(Table .7): CD4 count in HIV seropositive individuals.

<table>
<thead>
<tr>
<th>CD4 cell/ µl</th>
<th>Number of patients</th>
<th>percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 200 cell/ µl</td>
<td>18/30</td>
<td>60%</td>
</tr>
<tr>
<td>200-500 cell/ µl</td>
<td>10/30</td>
<td>33.3%</td>
</tr>
<tr>
<td>&gt; 500 cell/ µl</td>
<td>2/30</td>
<td>6.7%</td>
</tr>
</tbody>
</table>
Fig: 16 Frequency distribution of T.Bilirubin level in HIV seropositive individuals

Normal Values: (Up to 1 mg/dl)

Fig: 17 Frequency distribution of ALT level in HIV seropositive individuals

Normal Values: (Up to 40 IU/L)
Fig: 18 Frequency distribution of AST level in HIV seropositive individuals

Normal Values: (Up to 40 IU/L)

Fig: 19 Frequency distribution of ALP level in HIV seropositive individuals

Normal Values: (Up to 113 IU/L)
Fig: 20 Frequency distribution of T. Protein level in HIV seropositive individuals

Normal Values: (6.0 - 8.0 g/dl)

Fig: 21 Frequency distribution of Albumin level in HIV seropositive individuals

Normal Values: (3.5 - 5.0 g/dl)
Fig: 22 Frequency distribution of Urea level in HIV seropositive individuals

Normal Values: (10 - 50 mg/dl)

Fig: 23 Frequency distribution of Creatinine level in HIV seropositive individuals

Normal Values: (0.4 - 1.4 mg/dl)
Fig: 24 Frequency distribution of Calcium level in HIV seropositive individuals

Normal Values: (8.1 - 10.4 mg/dl)

Fig: 25 Frequency distribution of Sodium level in HIV seropositive individuals

Normal Values: (135 - 150 mmol/l)
Fig: 26 Frequency distribution of Potassium level in HIV seropositive individuals

Normal Values: (3.5 - 5.5 mmol/l)

(Table .8): Sex distribution of the three groups in the study population

<table>
<thead>
<tr>
<th>Group</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>VL</td>
<td>57/99 (57.6%)</td>
<td>42/99 (42.4%)</td>
</tr>
<tr>
<td>VL/HIV</td>
<td>5/7 (71.4%)</td>
<td>2/7 (28.6%)</td>
</tr>
<tr>
<td>HIV</td>
<td>23/30 (76.7%)</td>
<td>7/30 (23.3%)</td>
</tr>
</tbody>
</table>

(Table .9): Age distribution of the three groups in the study population

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean (years)</th>
<th>Range/years</th>
</tr>
</thead>
<tbody>
<tr>
<td>VL</td>
<td>16</td>
<td>2 – 75</td>
</tr>
<tr>
<td>VL/HIV</td>
<td>20</td>
<td>13 – 26</td>
</tr>
<tr>
<td>HIV</td>
<td>40</td>
<td>30 – 54</td>
</tr>
</tbody>
</table>
(Table .10): Mean of the Renal and liver profiles of the three groups in the study population.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>VL patients (mean value)</th>
<th>VL/HIV co-infected (mean value)</th>
<th>HIV patients (mean value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T.Bilirubin mg/dl</td>
<td>0.8</td>
<td>0.63</td>
<td>0.9</td>
</tr>
<tr>
<td>D.Bilirubin mg/dl</td>
<td>0.46</td>
<td>0.23</td>
<td>0.38</td>
</tr>
<tr>
<td>ALT U/L</td>
<td>32.2</td>
<td>43.4</td>
<td>66.8</td>
</tr>
<tr>
<td>AST U/L</td>
<td>124</td>
<td>138</td>
<td>62.9</td>
</tr>
<tr>
<td>ALP U/L</td>
<td>135</td>
<td>157</td>
<td>114.7</td>
</tr>
<tr>
<td>T.Protein mg/dl</td>
<td>7.8</td>
<td>7.5</td>
<td>7.5</td>
</tr>
<tr>
<td>Albumin mg/dl</td>
<td>3.2</td>
<td>3.3</td>
<td>3.2</td>
</tr>
<tr>
<td>Urea mg/dl</td>
<td>30</td>
<td>26</td>
<td>34</td>
</tr>
<tr>
<td>Creatinine mg/dl</td>
<td>0.83</td>
<td>0.71</td>
<td>0.99</td>
</tr>
<tr>
<td>Calcium mg/dl</td>
<td>8.2</td>
<td>8.1</td>
<td>8.0</td>
</tr>
<tr>
<td>Sodium mmol/l</td>
<td>127</td>
<td>121</td>
<td>133</td>
</tr>
<tr>
<td>Potassium mmol/l</td>
<td>4.0</td>
<td>4.0</td>
<td>3.9</td>
</tr>
</tbody>
</table>

3.4. Comparison between epidemiological, serological, renal and liver profiles of the three groups of study subjects:

For clinical and epidemiological data Chi square test was used to compare the difference between the three groups of study subjects (significant difference: P.Value less than 0.05) (Tables 11, 12). The T.Test was used to compare the means of the different biochemical parameters in the three groups (Significant difference P.Value less than 0.05). Significant differences were observed between VL and VL/HIV co infected cases in DAT titers, gender, age, ALT, AST, ALP, total protein, albumin, and creatinine and sodium levels. In addition, comparison
between VL/HIV co infected cases and HIV seropositive individuals showed significant differences in DAT titers, gender, age, tribe ,ALT ,AST , total protein , albumin, creatinine and sodium levels .

(Table .11): Comparison between epidemiological, serological ,renal and liver profiles of VL and VL/HIV co infected cases

<table>
<thead>
<tr>
<th>Parameter</th>
<th>VL patients</th>
<th>VL/HIV co-infected patients</th>
<th>P.Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAT Titre</td>
<td>10860.6</td>
<td>7314.3</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Gender</td>
<td>57 males/42 females</td>
<td>5 males/2 females</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Tribe</td>
<td>Masaleet</td>
<td>Masaleet</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Age/Years</td>
<td>16</td>
<td>20</td>
<td>0.00</td>
</tr>
<tr>
<td>T.Bilirubin mg/dl</td>
<td>0.8</td>
<td>0.63</td>
<td>0.94</td>
</tr>
<tr>
<td>D.Bilirubin mg/dl</td>
<td>0.46</td>
<td>0.23</td>
<td>0.63</td>
</tr>
<tr>
<td>ALT U/L</td>
<td>32.2</td>
<td>43.4</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>AST U/L</td>
<td>124</td>
<td>138</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>ALPU/L</td>
<td>135</td>
<td>157</td>
<td>0.02</td>
</tr>
<tr>
<td>T.Protein mg/dl</td>
<td>7.8</td>
<td>7.5</td>
<td>0.02</td>
</tr>
<tr>
<td>Albumin mg/dl</td>
<td>3.2</td>
<td>3.3</td>
<td>0.003</td>
</tr>
<tr>
<td>Urea mg/dl</td>
<td>30</td>
<td>26</td>
<td>0.4</td>
</tr>
<tr>
<td>Creatinine mg/dl</td>
<td>0.83</td>
<td>0.71</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Calcium mg/dl</td>
<td>8.2</td>
<td>8.1</td>
<td>0.6</td>
</tr>
<tr>
<td>Sodium mmol/l</td>
<td>127</td>
<td>121</td>
<td>0.001</td>
</tr>
<tr>
<td>Potassium mmol/l</td>
<td>4.0</td>
<td>4.0</td>
<td>0.52</td>
</tr>
</tbody>
</table>
(Table .12): Comparison between epidemiological, serological, renal and liver profiles of VL/HIV co-infected patients and HIV seropositive individuals.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>VL/HIV co-infected patients</th>
<th>HIV patients</th>
<th>P.Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAT Titer</td>
<td>7314.3</td>
<td>2720</td>
<td>0.00</td>
</tr>
<tr>
<td>Gender</td>
<td>5 males/2 females</td>
<td>23 males/7 females</td>
<td>0.04</td>
</tr>
<tr>
<td>Age/ Years</td>
<td>20</td>
<td>40</td>
<td>0.01</td>
</tr>
<tr>
<td>T.Bilirubin mg/dl</td>
<td>0.63</td>
<td>0.9</td>
<td>0.97</td>
</tr>
<tr>
<td>D.Bilirubin mg/dl</td>
<td>0.23</td>
<td>0.38</td>
<td>0.83</td>
</tr>
<tr>
<td>ALT U/L</td>
<td>43.4</td>
<td>66.8</td>
<td>0.02</td>
</tr>
<tr>
<td>AST U/L</td>
<td>138</td>
<td>62.9</td>
<td>0.00</td>
</tr>
<tr>
<td>ALP U/L</td>
<td>157</td>
<td>114.7</td>
<td>0.11</td>
</tr>
<tr>
<td>T.Protein mg/dl</td>
<td>7.5</td>
<td>7.5</td>
<td>0.02</td>
</tr>
<tr>
<td>Albumin mg/dl</td>
<td>3.3</td>
<td>3.2</td>
<td>0.07</td>
</tr>
<tr>
<td>Urea mg/dl</td>
<td>26</td>
<td>34</td>
<td>0.98</td>
</tr>
<tr>
<td>Creatinine mg/dl</td>
<td>0.71</td>
<td>0.99</td>
<td>0.003</td>
</tr>
<tr>
<td>Calcium mg/dl</td>
<td>8.1</td>
<td>8.0</td>
<td>0.67</td>
</tr>
<tr>
<td>Sodium mmol/l</td>
<td>121</td>
<td>133</td>
<td>0.001</td>
</tr>
<tr>
<td>Potassium mmol/l</td>
<td>4.0</td>
<td>3.9</td>
<td>0.52</td>
</tr>
</tbody>
</table>
4. Discussion

4.1 Visceral Leishmaniasis:

In the present study, a total number of 99 patients with VL were recruited from two kalazar centers in Gedarif and Sinnar states where the disease is endemic in Sudan since the beginning of 20th century (Zijlstra & Elhassan, 2001). As reported earlier both young and older children were equally affected with females being less affected. The clinical features were also similar to those reported by previous investigators. Fever, spleenomegaly and lymphadenopathy were found in all patients; other symptoms were present but were less common.

Investigations of Liver function parameters in the present study showed that hyperbilirubinemia, hypoalbuminaemia and an increase in liver enzymes were pronounced in the majority of VL patients. Most (85.9%) of the patients had elevated AST activity while elevated ALT and ALP were found in 19.2% and 55.6% respectively. However, only 9.1% patients were found to be jaundiced on examination. The majority (78.8%) of patients showed low albumin level and 40.4% had hyperproteinaemia. Our findings are in agreement with previous results reported by Mustafa et. al.(1995). However, hypoalbuminaemia can be explained by many other contributing factors mainly due to Nutritional habits, economical status, malaria and chronic diseases.

The laboratory results of the study subjects relating to the renal function tests indicated the prevalence of two cases of renal failure that had not been detected on clinical examination. Seven patients showed an increase in urea levels without deterioration in other renal function tests. Hyponatraemia was detected in 89(89.9%), hypocalcaemia in 48 (48.5%), hypokalama in 29(29.3%) and hyperkalaemia in 11(11.1%). These results are in accordance with another study.
done by (Daher et al. 2008) that indicated renal dysfunction in VL patients. Similar results were also reported by (Pintado et al. 2001). In addition, (Agenor Araujo Lima et al. 2009) reported on the functional tubular disturbances in Kala-azar cases. Their results showed that hyponatraemia were found in 94.6%, hypokalemia in 26% and hypocalcemia in 32%. Furthermore the renal involvement was documented in 15% of VL cases by (Prakash, et. al. 2007).

4.2 Leishmania/HIV co-infection:

The present study confirms that Leishmania/HIV co-infection is an emerging health problem in Sudan. The number of cases of Leishmania/HIV co-infection is expected to rise in the country owing to the simultaneous spread of the two infectious diseases and their increasingly overlapping geographical distribution, complicated by mass migration, displacement, civil unrest, and war. Seven (7.1%) of our patients were co-infected with HIV and VL. This figure is higher than was reported in earlier studies (Elsafi et al 1997); this may be explained by the fact that most of our VL patients were more than 16 years of age.

The parameters of liver function tests showed that 1 (14.3%) of the 7 co-infected cases had an elevated ALT, 7 (100%) had elevated AST activity, 6 (85.7%) had elevated ALP activity, 2 (28.6%) had hyperproteinemia and 5 (71.4%) had hypoalbuminaemia. Our results are in agreement with a previous study done in Italy by (Rollino et al., 2003).

The parameters of renal function tests indicated that 3 (42.9%) had hypocalcaemia, 7 (100%) had hyponatraemia, 2 (28.6%) had hypokalama and 1 (14.3%) had hyperkalaemia. Our results contradict the findings that were reported by (Rollino et al., 2003) in Italy. We explained that by severity of the disease and may be due to latency in the diagnosis of VL because that area was endemic with malaria and other chronic diseases had the same clinical symptoms.
4.3 HIV/AIDS:

The parameters of Liver and renal function tests were done for all HIV seropositive individuals. Hypocalcaemia was found in 16(53.3%); 3(10%) out of 30 were jaundiced, 12(40%) had elevated ALT, 11(36.7%) had elevated AST and the same number for ALP,. 6(20%) of the individuals had hyperprotiemaemia, 3(10%) had hypoprotienamia, 19(63.3%) had hypoalbuminaemia and 3(10%) had Azotamia. One (3.3%) had renal failure, 16(60%) had hyponatraemia, 2(6.7%) had hyperkalaemia, 10(33.3%) had hypokalamia, 13(43.3%) had hyperuricaemia and 1(3.3%) had hypouricaemia. Our results are comparable to the findings reported by Pintado et al., (2001).

In this study the CD4 count was done for all HIV patients during ART using flowcytometry. Our results indicated that the CD4 counts were < 200 cell/µl in the majority of patients and it was normal or subnormal in some patients who showed clinical improvement. Similar findings were reported in other studies that had been carried out in Sudan by (Magzoub et al., 2002) and in other countries by (Baker et al., 1998).

4.4 Comparative results:-

4.4.1 VL and VL/HIV co-infected patients:-

The comparison that was done between the two groups showed significant differences in epidemiological data (age and tribe) showed (P.Value < 0.05). The liver function tests also showed significant differences in ALT, ALP, AST, T.Protien and Albumin Levels (P.Value < 0.05) but there was no significant difference in T.Bilirubin and D.Bilirubin levels (P.Value > 0.05). The renal function tests relating to Creatinine and Sodium levels, were also significantly different between the two groups (P.Value < 0.05). However, there was no significant difference in Urea, Calcium and Potassium levels (P.Value > 0.05).
This may be due to latency in diagnosis or to the sequestration of the parasite in those tissues.

Finally there are no well planed studies addressing this problem in Sudan except liver function tests for VL patient that done by (Mustafa 1995). And also we recommend searching more in this area.
Conclusions & Recommendations

Conclusions:

1- Renal dysfunction is pronounced among VL patients, and can be useful for monitoring the disease severity and treatment.

2- Renal and liver profiles are variable among AIDS patients, depending on many contributing factors; including viremia, stage of disease, period of treatment and co-infection with other diseases.

3- Renal and liver profiles are variable among VL/HIV co-infected patients, also renal dysfunction is pronounced among them.

4- VL / HIV co-infection is an emerging health problem in the study (7.1%) which may be associated with a potential risk for more epidemics.
Recommendations:

1- The prevalence of Leishmaniasis among confirmed HIV patients is recommended to be further investigated using a larger sample size.

2- The renal and liver profiles should be investigated in VL cases when clinically indicated.

3- VL/HIV co-infection is expected to rise in the coming years, further studies are recommended to assess the magnitude of the problem at both hospital and community levels.

4- Development of effective health education program for HIV positive individuals in VL endemic areas.

5- Further research in the area of biochemical changes, CD4, CD8 count and parasite of VL, VL/HIV for molecular characterization.
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