1. Review of literature:

Literatures related to disease kala azar were reviewed from books, journals and electronic databases like HINARI, Pub med, MEDLINE, Cochrane library, Google scholar and search engine Google by using different descriptors. Visceral Leishmaniasis (VL), also known as kala-azar, black fever, and Dumdum fever is the most severe form of leishmanisis. Leishmaniasis is a disease caused by protozoan parasites of the Leishmania genus. This disease is the second-largest parasitic killer in the world (after malaria), responsible for an estimated 500,000 infections each year worldwide. The parasite migrates to the internal organs such as liver, spleen (hence ‘visceral’), and bone marrow, and, if left untreated, will almost always result in the death of the host. Signs and symptoms include fever, weight loss, muscular ulcers, fatigue, anemia, and substantial swelling of the liver and spleen. Of particular concern, according to the World Health Organization (WHO), is the emerging problem of HIV/VL co-infection. Two species of Leishmania are known to give rise to the visceral form of the disease. The species commonly found in East Africa and the Indian subcontinent is Leishmania Donovani and the species found in Europe, North Africa, and Latin America is Leishmania infantum also known as Leishmania chagasi. Visceral leishmaniasis (Kala-azar) is spread through an insect vector, the sandfly of the Phlebotomus genus in the Old World and the Lutzomyia genus in the New World. Sandflies are tiny creatures, 3–6 millimeters long by 1.5–3 millimeters in diameter, and are found in tropical or temperate regions throughout the world. Sandfly larvae grow in warm, moist organic matter (such as old trees, house walls, or waste) making them hard to eradicate. The adult female sandfly is a bloodsucker, usually feeding at night on sleeping prey. When the fly bites an animal infected with L. donovani, the pathogen is ingested along with the prey's blood. At this point the protozoan is in the smaller of its two forms, called an amastigote round, non-motile, and only three to seven micrometers in diameter. Taken into the stomach of the sandfly, the amastigotes quickly transform into a second L. donovani form, called the promastigote. This form is spindle-shaped, triple the size of the amastigote, and has a single flagellum that allows for mobility. The promastigotes live extracellularly in the sandfly's alimentary canal, reproducing asexually, then migrate to the proximal end of the gut where they become poised for a regurgitational transmission. This is their means of transmission back into a mammalian host, as the fly injects its saliva into prey when it bites. The promastigotes are introduced locally at the bite site along with the fly’s saliva.
Once inside the new host, promastigotes invade macrophages. Once inside, they transform back into the smaller amastigote form. As an amastigote, *L. donovani* can only reproduce intracellularly—and the amastigotes replicate in the most hostile part of the macrophage cell, inside the phagolysosome, whose normal defensive response they are able to prevent. After they have reproduced to a certain extent, the *L. donovani* lyse their host cell by sheer pressure of mass, but there is some recent speculation that they are able to leave the cell by triggering the exocytosis response of the macrophage. The daughter cell protozoans then migrate through the bloodstream to find new macrophage hosts. In time, *L. donovani* becomes a systemic infection, spreading to all the host's organs, particularly the spleen and liver.

**Disease progression:**

In human hosts, response to infection by *L. donovani* varies a great deal, not only by the strength but also by the type of the patient's immune reaction. People with a history of infection by strains of leishmania that cause visceral leishmaniasis show a continuum of immune responses from protective to non-protective. Those who acquired protective immunity (skin test positive) without ever having visceral leishmaniasis have a strong type 1 CD4+ response to leishmania antigens. Antigen specific interferon-gamma and proliferation, as well as the ability to kill intracellular leishmania, are hallmarks of protective immunity. Because visceral leishmaniasis patients lack these responses to leishmania and other antigens, they usually die of secondary infections unless treated. In addition, increased interleukin-10 secretion is characteristic of the disease. Addition of interleukin-12, anti-interleukin-10, or anti-interleukin-4 to peripheral blood mononuclear cells from acute patients sometimes increases interferon-gamma secretion and proliferation. Acute patient peripheral blood mononuclear cells include CD8+ T regulatory cells that decrease interferon-gamma secretion and proliferation responses to leishmania and other antigens and increase interleukin-10 secretion when added to autologous peripheral blood mononuclear cells harvested after successful treatment. Thus, the CD8+ T regulatory cells reproduce the immune response characteristic of visceral leishmaniasis. CD8+ T regulatory cells are also associated with post kala azar dermal leishmaniasis. Addition of interleukin-12 or interferon-gamma does not prevent CD8+ T regulatory activity. The dominance of type 1 CD4+ T cells in skin test positive adults may be explained by their secretion of factors that inhibit and kill CD8+ T regulatory cells. Successfully treated patients rarely develop visceral leishmaniasis a second time. Their peripheral blood mononuclear cells show a mixed T1/T2 CD4+ and CD8+ T suppressor response but do have the ability to kill intracellular leishmania.

When a human patient does develop visceral leishmaniasis, the most typical symptoms are fever and the enlargement of the spleen, or splenomegaly, with enlargement of the liver—
hepatomegaly—sometimes being seen as well. The blackening of the skin that gave the disease its common name in India does not appear in most strains of the disease, and the other symptoms are very easy to mistake for those of malaria. Misdiagnosis is dangerous, as without proper treatment the mortality rate for kala-azar is close to 100%. *L. donovani* itself is not usually the direct cause of death in kala-azar sufferers, however. Pneumonia, tuberculosis, and dysentery are omnipresent in the depressed regions where leishmaniasis thrives, and, as with ADIS, it is these opportunistic infections that are more likely to kill, flaring up in a host whose immune system has been weakened by the *L. donovani* infection. Progress of the disease is extremely variable, taking anywhere from one to twenty weeks, but a typical duration for the Sudanese strain of the disease is narrower, between twelve and sixteen weeks.

Even with recovery, kala-azar does not always leave its hosts unmarked. Sometime after successful treatment—generally a few months with African kala-azar, or as much as several years with the Indian strain—a secondary form of the disease may set in, called post kala azar dermal leishmaniasis or PKDL. This condition manifests first as small, measles-like skin lesions on the face, which gradually increase in size and spread over the body. Eventually the lesions may coalesce to form disfiguring, swollen structures resembling leprosy, and occasionally causing blindness if they spread to the eyes. This disease is not the same as cutaneous leishmaniasis a milder disease caused by another protozoan of the Leishmania genus which also causes skin lesions.

**Diagnosis:**

The gold standard for diagnosis is visualization of the amastigotes in splenic aspirate or bone marrow aspirate. This is a technically challenging procedure that is frequently unavailable in areas of the world where visceral leishmaniasis is endemic.

Serological testing is much more frequently used in areas where leishmaniasis is endemic. The K39 dipstick test is easy to perform, and village health workers can be easily trained to use it. The kit may be stored at ambient temperature and no additional equipment needs to be carried to remote areas. The DAT anti-leishmania antigen test is standard within MSF is much more cumbersome to use and appears not to have any advantages over the K39 test.

There are a number of problems with serological testing: in highly endemic areas, not everyone who becomes infected will actually develop clinical disease or require treatment. Indeed, up to 32% of the healthy population may test positive, but not require treatment. Conversely, because serological tests look for an immune response and not for the organism itself, the test does not become negative after the patient is cured, it cannot be used as a check for cure, or to
check for re-infection or relapse\textsuperscript{39}. Likewise, patients with abnormal immune systems (e.g., HIV infection) will have false-negative tests\textsuperscript{43}.

Other tests being developed include a Latex agglutination test (KA test), which is currently being tested in Asia and Africa. Another potential test detects erythrosalicylic acid\textsuperscript{39}.

**Treatment:**

As with many diseases in developing nations, (including trypanosomiasis and malaria) effective and affordable chemotherapy is sorely lacking and parasites or insect vectors are becoming increasingly resistant to existing anti-parasite drugs. Possibly due to the lack of financial return, new drugs are slow to emerge and much of the basic research into potential drug targets takes place in universities, funded by charitable organizations. This may change as a result of infection of members of the armed forces from the developed nations that currently occupy nations such as Afghanistan and Iraq, where *Leishmania* is commonplace.

The traditional treatment is with pentavalent antimonials such as Sodium Stibogluconate and meglumine antimoniate. Resistance is now common in India, and rates of resistance have been shown to be as high as 60\% in parts of India, Nepal and Bangladesh\textsuperscript{59,62}.

The Indian medical practitioner, Upendra Nath Brahmachari, was nominated for the Nobel Prize in Physiology or Medicine in 1929 for his discovery of ureastibamine (an antimonial compound for the treatment of kala-azar) and a new disease, post kala-azar dermal leishmaniasis. Brahmachari's cure for visceral leishmaniasis was the urea salt of para-amino-phenyl stibnic acid which he called Urea Stibamine. The treatment of choice for visceral leishmaniasis acquired in India is now Amphotericin B 25 in its various liposomal preparations (AmBisome\textsuperscript{63}, Abelcet, Amphocil\textsuperscript{58}). AmBisome dose: total dose 21 mg/kg (Mediterranean/Brazilian VL); total dose 7.5 mg/kg over 6 days (Indian VL). Amphocil dose: total dose 7.5 mg/kg over 6 days (Indian VL). A low dose (0.5–1 mg/kg) is given on the first day, increasing to 1–2 mg/kg on the second day, followed by 1.5–3 mg/kg on the third and subsequent days.

Miltefosine Impavido is the first oral treatment for this disease. The cure rate of miltefosine in Phase III clinical trials is 95\%; Studies in Ethiopia show that is also effective in Africa. In HIV immunosuppressed people, which are co infected with leishmaniasis it has shown that even in resistant cases 2/3 of the people responded to this new treatment. Miltefosine has received approval by the Indian regulatory authorities in 2002 and in Germany in 2004. It is now registered in many countries.

The drug is generally better tolerated than other drugs. Main side effects are gastrointestinal disturbance in the first or second day of treatment (a course of treatment is 28 days) which does
not affect the efficiency. Because it is available as an oral formulation, the expense and inconvenience of hospitalization is avoided, and outpatient distribution of the drug becomes an option, making Miltefosine a drug of choice.

The nonprofit Institution for one World Health has adopted the broad spectrum antibiotic paromomycin for use in treating VL; its antileishmanial properties were first identified in the 1980s. A treatment with paromomycin costs about $15 USD. The drug had originally been identified in the 1960s. The Indian government approved paromomycin for sale and use in August 2006.

In 2009, the Hebrew University of Jerusalem Kuvin Center for the Study of Infectious and Tropical Diseases, in a collaborative effort with Addis Ababa University, was awarded a grant by the Bill & Melinda Gates Foundation for research into visceral leishmaniasis in Ethiopia. The project will gather data to be analyzed to identify the weak links in the transmission cycle and devise methods for control of the disease.

Combination drug therapies are currently under investigation, particularly by the Drugs for Neglected Diseases initiative (DNDi). Combination therapies allow for the use of existing drugs in combination, each in lower doses, which helps to decrease the incidence of severe side effects and drug toxicity, as well as the risk for development of resistance against the drugs; they have been shown to be cost-effective strategies strategies. Comparative homology modeling of the enzyme Hypoxanthine-guanine phosphoribosyl transferees (HGPRT; EC 2.4.2.8) in L. donovani suggest that among all of the computationally screened compounds, pentamidine, 1,3-dinitroadamantane, acyclovir and analogs of acyclovir had higher binding affinities than the real substrate (guanosine monophosphate). The drug development pipeline is lacking significantly, and no novel drug targets are expected for approval in the next 5 years. In the meantime, new combination therapies, and well as improvements to existing drugs targets, are under development. Single-dosage administrations of liposomal amphotericin B have been shown to be effective, and oral formulations are currently under development to increase access and facilitate distribution of the efficacious drug in the field.

**Vaccine:**

There is no vaccine for this disease as of 2014. On May 2012, the nonprofit Infectious Disease Research Institute launched the world’s first clinical trial of the visceral leishmaniasis vaccine. The vaccine is a recombinant form of two fused Leishmania parasite proteins with an adjuvant. Two phase 1 clinical trials with healthy volunteers are to be conducted. The first one takes place in Washington (state) and is followed by a trial in India.
The use of lipid complexes amphotericin b to treat visceral leishmaniasis was pioneered by Henry Murray, MD, who recognized that infected macrophages would engulf the lipid complexes, liberating amphotericin at the point of infection while minimizing untoward systemic side effects, e.g. renal distal tubule damage. Doctor Murray's work resulted in a paradigm shift in the treatment of and control of this parasitic disease in India.

The first ever vaccine to prevent visceral leishmaniasis (VL), the second-largest parasitic killer in the world after malaria, is entering dual clinical trials in the US and India in 2013. The Infectious Disease Research Institute (IDRI) vaccine, called LEISH – F3 + GLA – SE, is a highly purified, recombinant vaccine which incorporates two fused Leishmania parasite proteins.

History and epidemiology

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. Another common name, kala-azar (Hindi: काला आजार,  کالا آزار (Nastaleeq) kāḷā āzār), is derived from kala which means black in Sanskrit, as well is the languages descended from it, including Assamese25, Hindi and Urdu the word azar means disease in Persian and Hindustani26 as such the disease is named for the darkening of the skin on the extremities and abdomen that is a symptom of the Indian form of the disease. The agent of the disease was also first isolated in India by Scottish doctor William Leishman and Irish physician Charles Donovan, working independently of each other. As they published their discovery almost simultaneously, the species was named for both of them—Leishmania donovani.

Today, the name kala-azar is used interchangeably with the scientific name visceral leishmaniasis for the most acute form of the disease caused by L. donovani. The disease is endemic in West Bengal, where it was first discovered, but is seen at its most deadly in north and east Africa. It can also be found throughout the Arab world and southern Europe (where the causative organism is L. infantum), and a slightly different strain of the pathogen, L. chagasi, is responsible for leishmaniasis in the new world. Several species of canines serve as reservoir hosts of L. infantum (chagasi).

But, while the disease's geographical range is broad, it is not continuous. The disease clusters around areas of drought, famine, and high population density. In Africa, this has meant a knot of infection centers mostly in Sudan, Kenya and Somalia. Living conditions here have changed very little in the past century, and the people are not normally very mobile. Parts of the Sudan, in particular the Upper Nile region, are almost totally cut off from the rest of the country, and the people are as tied to the place of their birth as any peasant of Europe’s Dark Ages33.
Contemporary life has made itself felt even here, however—not as "progress" but in the form of the many small wars of Africa's post-colonial era. In the Sudan, where civil war has been continuous since 1983, the violence has been concentrated in the more populated south, and kala-azar was concentrated there too. But the wars have driven a steady stream of refugees out of the region, and these traveled either across the southern border or into the remoter western part of the country called the Upper Nile, where both war and the disease that went with it had not yet penetrated. Now India, Nepal and Bangladesh are is the endemic zone of this disease.
Associated risk factors of kala azar:

A study carried out in Bihar, India between March 1st, 2007 and December 1st, 2008 was found that no significant associations between VL and keeping domestic animals inside the house (OR of 0.88 for bovines and 1.00 for 'any animal') or ownership of domestic animals (OR of 0.97 for bovines and 1.02 for 'any animal'). VL was associated with housing conditions. Living in a thatched house (OR 2.60, 95% CI 1.50-4.48) or in a house with damp floors (OR 2.60, 95% CI 1.25-5.41) were risk factors, independently from socio-economic status. A case control study in eastern Nepal found that cases were strongly clustered, 70% residing in 3 out of 19 neighborhoods and a strong association with socio-economic status, the poorest being most at risk. Housing was a risk factor independent from socio-economic status, most at risk were those living in thatched houses without windows. 'Sleeping upstairs' and 'sleeping on a bed' were strongly protective, OR of 0.08 and 0.25 respectively; proximity to a case was a strong risk factor (OR 3.79). A study on the risk factors associated with kala-azar in disease-endemic areas of Bihar, India, a univariate analysis showed that education, a history of other diseases in the previous year, a history of kala-azar in the family, type of walls in houses, presence of a granary inside houses, presence of vegetation around houses, bamboo trees near houses, and irregular spraying around houses with DDT were risk factors. Multivariate analysis showed that a history of other diseases in the previous year (odds ratio [OR] = 3.6, P = 0.002), a history of kala-azar in the family (OR = 1.8, P = 0.03), mud-plastered walls in houses, (OR = 2.4, P = 0.0001), a granary inside houses (OR = 4.3, P = 0.0001), presence of bamboo trees around houses (OR = 2.3, P = 0.001), and houses not sprayed with DDT in the past six months (OR = 3.4, P = 0.0001) were significant risk factors for kala-azar. A study on children in high incidence sub-districts of Amhara regional state, Ethiopia showed that the individual variables that showed a positive association with infection were increasing age, being male and sleeping outside [adjusted odds ratios (95% CI): 1.15 (1.03, 1.29), 2.56 (1.19, 5.48) and 2.21 (1.03, 4.71) respectively] and in relation to the household: past history of VL in the family, living in a straw roofed house and if the family owned sheep [adjusted OR (95% CI): 2.92 (1.25, 6.81), 2.71 (1.21, 6.07) and 4.16 (1.41, 12.31) respectively]. A behavioural pattern like sleeping outside is determinant in the transmission of the infection in that area. Protective measures should be implemented against that identified risk activity. A cross-sectional study was conducted to evaluate the risk factors for kala azar in Raposa municipality is an endemic area in State of Maranhão, Brazil, showed that the variables associated with infection upon nonadjusted analysis were a straw roof, mud walls, floors of beaten earth, presence of sand flies inside or outside of the dwelling, and bathing outdoors. Adjusted analysis showed that the presence of sand flies inside/outside the dwelling was a risk factor, and age younger than 10 years was a protective factor against asymptomatic
infection. The results highlight the extent to which precarious living conditions of the population strengthen the epidemiological chain of visceral Leishmaniasis. A study was carried out in Bihar State, India to identify factors associated with incidence of visceral leishmaniasis (VL). For this purpose they surveyed 13,416 households and found that VL was associated with socioeconomic status, type of housing, and belonging to the Musahar caste. Annual coverage of indoor residual insecticide spraying was 12%. Increasing such spraying can greatly contribute to VL control.

A review of studies published in the period 2005-2010 on the efficacy of different tools to control Phlebotomus argentipes. The Visceral Leishmaniasis (VL) Elimination Initiative in the Indian subcontinent was launched in 2005 as a joint effort between the governments in the Region (India, Nepal and Bangladesh) and the World Health Organization (WHO). The review indicates that the current indoor residual spraying (IRS) and novel vector control methods mainly insecticide treated nets (ITN) have low effectiveness for several reasons. Efforts to improve quality of IRS operations and further research on alternative and integrated vector control methods need to be promoted to reach the VL elimination target by 2015. A study estimated that over 1000 million people currently suffer from one or more Neglected Tropical Disease (NTD). The socio-economic aspects of two NTD - human African trypanosomiasis and human visceral leishmaniasis - are reviewed. Both of these diseases affect the poorest of the poor in endemic countries, cause considerable direct and indirect costs (even though the national control programmes tend to provide free care) and push affected households deeper into poverty. Study was conducted in highly prevalent area of Visceral Leishmaniasis is in Bihar, India showed that women had a significantly lower prevalence than men >14 years old. Owning domestic animals (cows, buffaloes or goats) was associated with a higher risk of being DAT positive [OR 1.16 (95% CI 1.01-1.32)], but socio-economic status was not. Although a study showed that Visceral leishmaniasis clearly affects the poorest of the poor in India. They are most vulnerable, as this vector-born disease is linked to poor housing and unhealthy habitats. The disease leads the affected households to more destitution because of its impact on household income and wealth. Support for the present VL elimination initiative is important in the fight against poverty. A random cross-sectional population survey was conducted in two visceral leishmaniasis (VL) foci in Morang District of Nepal in April to May 2003. Independent risk factors for Leishmania infection were proximity of the house to ponds [odds ratio (OR) 3.7, 95% CI: 1.6-8.5], family size (OR 4.4, 95% CI: 1.6-12.6), age > or =15 years (OR 5.5, 95% CI: 1.2-25.0) and house
constructed in mud (OR 3.0, 95% CI: 1.1-7.6). Bednets, not impregnated and in poor condition, were used by 95.2% (95% CI: 92.3-97.0) of the population, but did not show any protective effect. To determine the up-to-date morbidity and mortality trend for VL in Nepal data collected from eight zonal hospitals in the Terai region suggests that the first confirmed case of VL was recorded in 1980. By 2003, the disease has spread to 14 districts of central and eastern regions of Nepal, and nearly six million people residing in these districts were at the risk of acquiring the disease. A total of 25890 cases with 599 deaths were reported during the year 1980-2006 (up to July). The case fatality rate (CFR) varied from 0.23% to 13.2%. The highest incidence (per 100,000) was in Mahottari district (184), followed by Sarlahi (100) and Sunsari (96). The highest CFR was in Dhanusha (2.9%) followed by Bara (2.4%) and Saptari (2.0%). Majority (70.9%) of persons affected by VL were aged 15 years and above, followed by 10-14 years (13.9%), 5-9 years (11.9%) and 1-4 years (3.3%). The incidence of VL in Nepal seems to be increasing at a faster rate indicating that the existing control programs have been ineffective. Public health education, to make the people aware about preventive aspects of the disease is important. The possibility of the existence of animal reservoirs should also be considered and checked out for better control measures. A study in 2004, a cluster of kala-azar cases in Chatrakhali, West Bengal, India showed that factors associated with infection included residing in homes with mud walls (RR 4.3), dampness in the home (RR 2.5), proximity to bodies of water (RR 2.5) and livestock ownership (RR 2.4). Sleeping dressed (RR 0.4), or under a bed net (RR 0.5) or in a cot (RR 0.6) were associated with a lower risk. High rates of infection indicated that transmission persisted in this community. Poor housing conditions were associated with a higher risk, while personal protection measures against vectors were effective. Major housing improvement and personal protection efforts are needed to protect this vulnerable population from Leishmaniasis. A cross-sectional surveys over a 3-year period in a Bangladeshi community showed that risk factors for kala-azar was highest for persons 3-45 years of age, and no significant difference by sex was seen. In age-adjusted multivariable models, 3 factors were identified: proximity to a previous kala-azar patient (odds ratio [OR] 25.4, 95% confidence interval [CI] 15-44 within household; OR 3.2 95% CI 1.7-6.1 within 50 m), bed net use in summer (OR 0.7, 95% CI 0.53-0.93), and cattle per 1,000 meter square (OR 0.8, 95% CI 0.70-0.94). No difference was seen by income, education, or occupation; land ownership or other assets; housing materials and condition; or keeping goats or chickens inside bedrooms. Our data
confirm strong clustering and suggest that insecticide-treated nets could be effective in preventing kala-azar\textsuperscript{7}. A survey was conducted two villages within the Saran district of Bihar, India, and results indicated that men contracted the disease more than women (58\%), and cases over the age of 21 years accounted for 42\% of the total VL cases. April to June showed the highest number of new cases. Of 135 households surveyed for sleeping conditions, 95\% reported sleeping outside, and 98\% slept in beds. Proximity to VL cases was the greatest risk factor (cluster 1 relative risk = 11.89 and cluster 2 relative risk = 138.34).\textsuperscript{44}