

CDAlert

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LEISHMANIASIS AND ITS CONTROL

INTRODUCTION

Leishmaniasis is a disease caused by protozoan parasite of the genus *Leishmania* and transmitted by a phlebotomine sandfly. The various forms of leishmaniasis are: -

- Visceral leishmaniasis (VL), usually fatal when untreated.
- Cutaneous leishmaniasis (CL), a disabling disease when lesions are multiple.
- Muco-cutaneous leishmaniasis (MCL), a mutilating disease, and
- Diffuse cutaneous leishmaniasis (DCL), a disabling disease.

It is a worldwide disease, affecting 88 countries, and ninety percent cases of VL occur in 5 countries: India, Bangladesh, Nepal, Sudan and Brazil. As per WHO estimate visceral leishmaniasis (Kala-azar) affects 5,00,000 population globally of which 50% cases are from Indian subcontinent and India contributes the bulk of the reported VL cases and very few cases of cutaneous leishmaniasis.

In India, two types of leishmaniasis i.e. visceral leishmaniasis & cutaneous leishmaniasis are prevalent.

VISCERAL LEISHMANIASIS

- Visceral leishmaniasis or Kala-azar known to occur in India was first noticed in 1820s in undivided Bengal and often confused with malaria.
- The disease had almost disappeared from the country in 1950s due to the collateral benefit of DDT spray under National Malaria Eradication Programme (NMEP).
- Resurgence was reported in 1970s, when about 1,00,000 cases and 4,500 deaths were estimated from North Bihar and thereafter the disease had spread to adjoining States of West Bengal and Uttar Pradesh.
- About 350 million people are exposed to the risk of visceral leishmaniasis in endemic countries and 10 million are affected with the disease (WHO Technical Report series).
- Presently 31 districts in Bihar, 11 districts in West Bengal, 4 districts in Uttar Pradesh and 4 districts in Jharkhand are reporting cases of visceral leishmaniasis or Kala-azar. Total cases and deaths reported by these states during 2000-2005 are given in table below.
- Bihar alone contributes about 70-75% cases

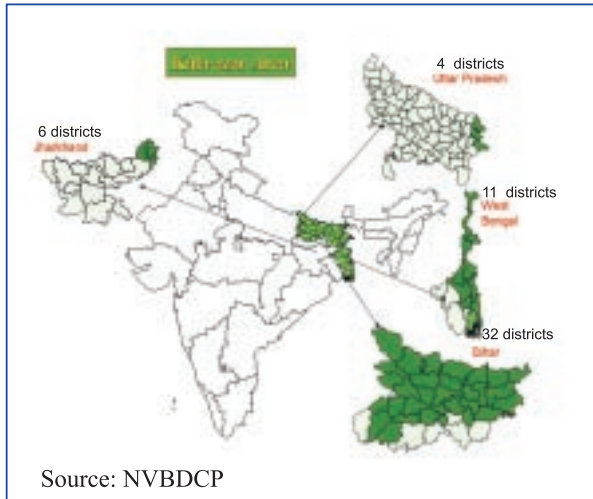
Table - Kala-azar cases and deaths reported during 2000-2005 (Source: NVBDCP)

Year	Bihar		West Bengal		Jharkhand		Uttar Pradesh		India*	
	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths
2000	12909	130	1244	11	469	0	47	0	14669	141
2001	10327	204	1238	4	589	0	22	3	12239	213
2002	9684	160	1592	5	758	0	32	1	12140	168
2003	13960	187	1487	7	2607	5	34	1	18214	210
2004	17324	107	3015	23	4028	14	34	2	24479	155
2005	21797	124	2706	15	6578	12	73	2	31217	157

*Data include imported cases reported from Delhi, Gujarat, Assam

and majority of deaths recently reported from India.

- Various types of PKDL lesions reported are: Macular, Maculopapular, Nodular and pigmented patches.
- In the neighboring countries disease is prevalent in Bangladesh, and Nepal.



VISCERAL LEISHMANIASIS

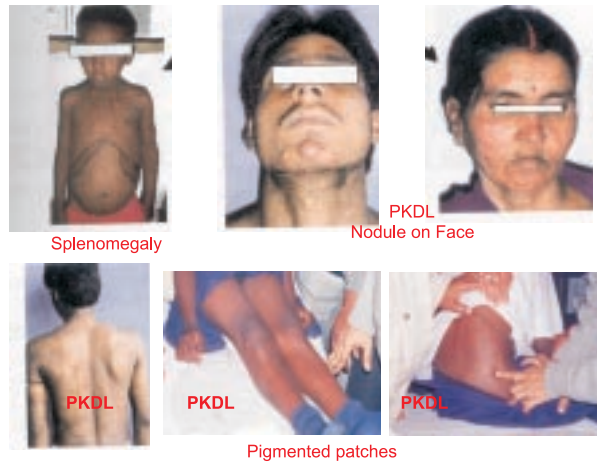


Fig. 1

reported from Tibbi area of Hanumangarh district Bikaner (Rajasthan).

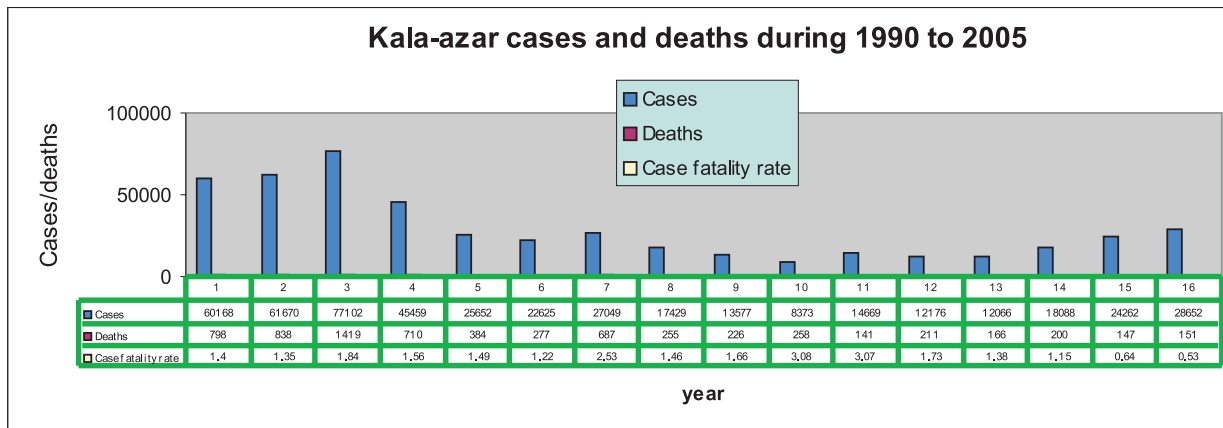
- Sporadic cases of CL were also reported from Fazilka (Punjab), Jodhpur city, rural & urban areas of Bikaner (Rajasthan).
- Suspected cases of CL were reported from Koyallam district of Kerala during 2001-2003.
- CL was not known to occur in Himachal Pradesh prior to 1988. In 1988, the first case of CL was reported by Indira Gandhi Medical College, Shimla (H.P.) and since then cases of CL are on rise.
- The cases of CL reported by Medical College hospital in Shimla (H.P.) are as under:

1988-2002	22 cases
2003	101 cases
2004	70 cases upto September
- Cases were found with 1-3 lesions on face, neck, chest and extremities.

CUTANEOUS LEISHMANIASIS

Commonly known as Delhi boil or Oriental sore. In India, indigenous cases of cutaneous leishmaniasis (CL), anthroponotic cutaneous leishmaniasis (ACL) as well as zoonotic cutaneous leishmaniasis (ZCL) are mainly confined to hot dry northwestern region and are endemic in the western Thar Desert of Rajasthan.

- With the spray of DDT under NMEP, cases of CL were almost non-existent.
- In 1976, a severe outbreak from Bikaner town was reported, wherein > 2000 cases occurred & till eighties cases of CL were



VECTORS OF LEISHMANIASIS (SANDFLIES)

Sandflies are extremely small, fuzzy, delicate insects, usually 1/3rd of the size of mosquitoes. The length of the body ranges from 1.5 -3.5 mm, so they can easily pass through an ordinary mosquito net.

Six genera of sandflies are reported from the world however, in the Indian subcontinent, only two genera i.e. *Phlebotomus* and *Sergentomyia* are found.

Vector of visceral leishmaniasis

- *P. argentipes* is the sole vector of kala-azar in India.
- It is widely distributed in Sri Lanka, Bangladesh and Nepal and is found abundantly in warm and moist climate. It is also found in Pakistan, Myanmar, Thailand, Malaysia, China and Indonesia.
- *P. argentipes* has been reported upto a height of 1200M above mean sea level (MSL) in Kasauli & Nilgiri hills & 1300 MSL in Himalayan Garwal region.

Vectors of cutaneous leishmaniasis

- *P. papatasi*, recorded from all regions of country except north-eastern areas and Kerala. Rarely found on western coast and absent from the Himalayan states.
- *P. sergenti*, recorded from western parts of India, Thar Desert, northern plains. Urban species confined to North-West India.
- *P. salehi*, recorded in small part of Thar desert of Rajasthan
- Vectors of CL are more common in the arid part of north-west India.

LIFE CYCLE

Life cycle of sandfly includes four stages : egg, larva, pupa and adult (Fig.-2).

Egg: A female sandfly may lay about 50-60 eggs per oviposition on soil, in cracks /crevices and microclimatic conditions with moisture and decaying organic matter with high humidity. Eggs may hatch in about 3-6 days.

Larva: Freshly emerged larva is creamy white having hairs on its body. It is a voracious feeder & feeds on the organic matter present in the

soil. Upon feeding the 1st stage larva moults 3 times to form 4th stage larva. The developmental period would depend on the availability of food, temperature & humidity conditions. The larva may take about 2 weeks to transform into a pupa.

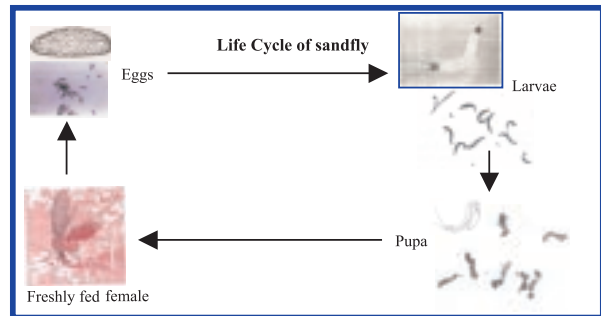


Fig. 2

Pupa: Sandfly pupa is elongated comma shaped, non-feeding, non-motile stage. The terminal end of the pupa remains attached with skin of 4th stage larva. The pupal period is about one week under normal conditions.

Adult: The development from egg to the emergence of adult takes about four weeks. Adults are found in cracks & crevices in dark corners of house/cattle sheds, caves, animal burrows, termite mounds, tree holes and culverts. Adults are crepuscular in habit and generally endophilic in nature.



Female *P. argentipes*

Male *P. argentipes*

FIELD SAMPLING FOR VECTOR SURVEILLANCE

Sampling of sandflies is essential to determine the species, their distribution, population dynamics, and in vector surveillance.

Sampling of adults:

Hand collection

Sandflies resting on different surfaces (indoor or outdoor) can be collected by using an aspirator tube or test tube and torchlight. Result is expressed in term of per man-hour density

(PMHD) and method is very effective for evaluation of sandfly density. Sampling of resting population of sandflies in diverse habitats, viz., trunks of trees, tree holes, caves, rodent borrows, stables, chicken houses and bedrooms can also be done by this method.

Sticky traps

Sticky traps have extensively been used for comparing densities of sandfly species in different habitats and seasons. The sandflies sit frequently on randomly placed sticky traps and are trapped. Standard A4 size sheets of paper (20 x 30 cm) after smeared with castor oil on both the sides are extensively used in these sandfly traps. Traps are placed during evening hours in suspended arched position with convex side towards ground placed at a height of 4-5 cm from the ground. The traps are generally placed near the soil cracks, rodents burrow, ground of the forest, grasslands, trees and bushes, on the wall in houses/ cattle sheds and stables. In the next morning flies are picked up from the oiled paper with the help of a camel hair brush and preserved in 70% alcohol for identification in the later stage. The density is recorded as total sandflies collected per night per trap. Twenty such traps give statistically valid result in each locality.

VECTOR BIONOMICS

Resting habits

Sandflies are crepuscular in habit; they remain inactive during daytime and rest in cracks and crevices in the dark corners of house and cattle sheds. In indoor situations, they may rest in outbuildings, caves, crevices, animal burrows, termite hills and tree holes etc. *P. argentipes* is generally found in mud-plastered houses and cattle shed having humidity 80-85 percent, and temperature 25-30 °C. *P. papatasi* is endophilic in nature and *P. salehi* is exophilic.

Feeding habits

- Sandflies are zoophilic and their feeding habits change as per their resting behaviour.
- Female *Phlebotomus* mainly feed on wide variety of hosts but prefer cattle. However, it also feed on human beings frequently when they are present in human dwellings.

Breeding habits

- Sandfly breeds in loose soil with moisture and organic debris.
- In endemic areas the immature stages of sandflies are difficult to find and may be located in loose soil, cracks, crevices and closely packed earth in and around the basement of the mud wall shelters. Larvae of sandflies are notoriously difficult to find.
- Garbage/cow dung disposal site around houses are the excellent breeding conditions for sandflies.



Suitable Breeding conditions for Kala-azar vector

Flight range and dispersal

- Sandflies are short fliers and move by hopping movements.
- Maximum distance covered in a single hop is 1 to 1.5 feet.

Seasonal prevalence

The seasonal prevalence of sandflies varies from area to area depending upon the environmental factors.

- In Kala-azar endemic areas of Bihar and West Bengal there is continued presence of *P. argentipes* throughout the year with low density during winter and summer months.
- In foot hills of Nilgiris (T.N.) with no distinct cold season, *P. argentipes* is commonly found throughout the year.

Transmission cycle:

Kala-azar is transmitted by the bite of infective vector sandfly, *P. argentipes*. Sandfly may obtain parasite directly from the infected skin of PKDL cases or by ingesting parasites from the circulating blood of the human reservoir host. After taking the infected blood meal, the sandfly

becomes infective in 6-7 days under optimum conditions of temperature and humidity.

Incubation period of Kala-azar in man

- The incubation period of Kala-azar is usually 8-10 months.
- It may be as short as 10 days & can be prolonged upto 2 ½ years and even 30 years.

DIAGNOSIS OF LEISHMANIASIS

The diagnosis of leishmaniasis consists of demonstration or isolation of the parasite from blood or biopsy material and demonstration of Leishmania specific antibodies in the serum. In addition there are a few non-specific tests based upon the deviations in the normal blood picture and the serum proteins, which may also aid in diagnosis.

Collection, Storage and transportation of specimen

The collection, transportation and storage of specimens are extremely vital steps in laboratory diagnosis and hence, must be undertaken with utmost care.

- Collect sufficient quantity of specimen
- Avoid contamination by using sterile equipment and aseptic precautions.
- Despatch the specimen immediately to laboratory
- In case the delay is inevitable, keep the specimen at +4°C in a refrigerator.
- Label all specimens accurately and send all pertinent information to laboratory which will help in better interpretation of the laboratory findings.

Specimen

The following clinical specimens have to be collected:

- Blood (serum)
- Biopsy/Aspirate from - Bone marrow and/or spleen. Rarely biopsy of lymphnode may also be taken.

Blood

It can be collected either through a venipuncture or by finger prick method. By venipuncture 4-5 ml blood should be collected in a plain vial. Allow it to clot and separate the serum.

By finger prick method blood is collected on filter paper. It carries the advantage of easy transportation and requires less storage space.

Bone Marrow Aspirate

It can be collected from

- Mid - sternal region, a little away from the mid line at the level of second or third intercostal space.
- Posterior iliac crest puncture, 1 cm below the superior iliac spine.
- Bone marrow sample to be collected by clinician.

Spleen Puncture

When spleen is considerably enlarged it is one of the most valuable method for establishing the parasitological diagnosis of kala-azar.

Demonstration of the parasite

The conclusive evidence in the diagnosis of kala-azar is the demonstration of the parasite. It can be achieved in either of the following ways:

- **Microscopic examination of the stained film:** Make smears from bone marrow/ splenic aspirate and stain with Leishman or Giemsa stain. Examine for amastigote forms (L.D. bodies) of the parasite
- **Culture of the parasite:** Inoculate bone marrow or splenic aspirate in culture media like Tobies medium, NNN medium and incubate at 22 - 25°C for 3 - 4 days.

Specific Serological Tests

The specific antibodies to Leishmania can be demonstrated by following methods:

- Indirect Fluorescent Antibody Test (IFAT)
- Enzyme Linked Immunosorbent Assay (ELISA)
- Direct Agglutination Test (DAT)
- rK - 39 strip test (Commercially available)

The above tests are rapid, sensitive and specific and become positive very early in disease.

Indirect Evidences

Peripheral smear

Leucocyte count reveals leucopenia (neutropenia) with marked diminution of neutrophil granulocytes accompanied by a relative increase of lymphocytes and

monocytes. Eosinophil granulocytes are absent. During the course of the disease there is a progressive diminution of leucocyte count falling to 1000/cmm of blood or even below that.

Erythrocytes also decrease in number. The proportion of leucocytes to erythrocytes is greatly altered and may be about 1:2000 to 1:1000 (Normal 1:750).

Non - specific serological tests

Different tests employed are:

- Aldehyde test
- Antimony Test

A major disadvantage with these tests is their positivity in many other diseases where albumin: globulin ratio is reversed and are positive only when the disease is of 3 months duration.

Molecular Techniques

DNA probes and Polymerase Chain Reaction (PCR) can be used to detect and identify small numbers of parasites in tissues from infected humans. These methods are available only at few reference laboratories.

CONTROL MEASURES

Susceptibility status to insecticides

- *P. argentipes* is susceptible to DDT and other commonly used insecticides in most of the areas.
- *P. papatasi* is resistance to DDT and susceptible to Malathion and other insecticides in Bihar.

Prophylactic measures

It includes self-protection by the use of bed nets and use of repellents to prevent sandfly bite. For sandflies, net should be of approximately of 36-42-mesh size to prevent the entry of sandflies. Deet, DMP and citronella oil are effective compounds to be used as repellent.

Environmental measures

This method is applied to control/eliminate breeding sites of sandflies in and around houses by improving living conditions. This could be achieved by plastering the walls, filling up of the cracks/crevices with mud. Breeding of sandflies could also be controlled by proper sanitation.

Chemical control

As per the NVBDCP guidelines DDT (50% wp) is being used as residual spray for the control of *P. argentipes* in Kala-azar endemic areas. Two

rounds of indoor residual spray is undertaken in endemic areas @ 1 gm per sq. meter upto a height of 6 feet from ground level.

NATIONAL KALA-AZAR ELIMINATION PROGRAMME (Source NVBDCP)

- After the resurgence, cases and deaths due to Kala-azar continuously increased since early seventies. Through the centrally sponsored control programme of the Govt of India and its intensification during 1991, there was drastic reduction in mortality and morbidity due to Kala-azar from 77102 cases & 1419 deaths in 1992 to 45459 cases & 710 deaths in 1993.
- Based on the recommendation of the expert committee, Govt. of India has now approved 100 percent central assistance for Kala-azar elimination since December 2003 with the following strategy for its elimination by 2010.
 - ✓ Early diagnosis & complete treatment of kala-azar cases through primary health care system.
 - ✓ Vector control by undertaking residual insecticide spraying of houses and cattle sheds in the affected villages.
 - ✓ IEC activities to involve the community in the diseases control measures.
 - ✓ Training to upgrade technical skill of functionaries at various levels, in the treatment & the control of the diseases.
 - ✓ Regular monitoring of the programme.

Programme based activity & future plan

Expert committee: The meeting of expert committee to review strategies for kala-azar elimination was held on 21.04.2005. The meeting re-affirmed the commitment of goal of kala-azar elimination by 2010. Meeting emphasized that currently developed tools of diagnosis, treatment, vector control and opportunities available under Rural Health Mission makes it feasible to achieve elimination target. Some of the recommendations of the committee were as under:

1. Introduction of sero-diagnostic tools like, rk 39 and DAT.
2. Feasibility of incorporating oral drug Miltefosine as first line drug for treatment of kala-azar cases. This drug has already been registered with drug control of India and Phase IV trial has been completed.
3. Adequate coverage and quality of IRS with proper supervision and monitoring.

4. COMBI for Behavioral Change Communication.
5. Constitution of expert Group for diagnosis, treatment and Behavioural Change Communication.

GUIDELINES FOR USE OF RAPID DIAGNOSIS KIT rK39 (Source NVBDCP)

The diagnostic sensitivity of splenic aspiration is high (95%-98%), but the procedure carries a risk of bleeding; the sensitivity of examination of bone marrow specimens is considered to be lower (53%-95%). Organ aspiration and accurate examination of smears also require technical skills that are not uniformly available in rural areas. In the Kala-azar endemic areas of India, Napier's aldehyde test has been used for a long time.

Rapid diagnostic test (rK39)

A rapid dipstick test based on the recombinant K39 protein is available for rapid diagnosis of kala-azar. K39 is an epitope apparently conserved on amastigotes of Leishmania species that cause visceral infection. By use of laboratory ELISA testing, circulating anti-K39, IgG is detectable in 95% -100% of patients who have kala-azar, irrespective of geographic region. Using rK39 antigen-impregnated nitrocellulose strips developed for field conditions, finger prick-obtained blood and serum samples tested from Indian subjects demonstrated a sensitivity of 100% and a specificity of 97%. The strip testing yields results within five minutes.

TREATMENT OF VISCERAL LEISHMANIASIS (Source NVBDCP)

First Line of Treatment - once diagnosed as kala-azar and a patient receiving sodium stibogluconate outside without response:

- SSG non-responsive: No response to supervised SSG in 20 days and in areas of partial response in 30 days and/or two courses of SSG in fresh cases, start second line of treatment.
- Post Kala-azar Dermal Leishmanoid: 4 to 6 courses of SSG each comprising of 20 days as per the response with 10 days interval in the courses.

Side effects : Liver and cardiac toxicity

First Line of Treatment

Sodium Stibogluconate	20 mg/kg body weight (maximum 850 mg/day) by single injection.
Route	Intra-muscular (IM) at peripheral level i.e. below district and IM or Intravenous (IV) at district level and above
Duration	20 days, if partial response to 20 days treatment, then continue upto 30 days. Check for parasite load in splenic or bone marrow smear at 20 days or 30 days as the case may be.
Criteria for cure	Absence of L.D. bodies in aspirated material
Contraindication	Severe kidney, liver and heart disease
Precautions	Make drugs like Adrenaline, hydrocortisone hemisuccinate and other resuscitative measures available to guard against hypersensitivity reactions, etc.
Pregnancy	SSG should be given considering the danger to the mother if not treated as above

Second Line of Treatment

Amphotericin-B	1 mg per kg. body weight alternate days for fifteen days
Routes	Intravenous infusion in 5 per cent dextrose after mixing the drug in water for injection, very slowly in 6 to 8 hours
Criteria for cure	Absence of leishmania amastigotes bodies in aspirate material after 6 weeks and 6 months of the last dose
Contraindication	Kidney disease, severe liver and heart disease
Precautions	Stop the drug when signs of renal failure and those of hypokalaemia appear. Make available emergency drugs as I SSG to guard against hypersensitivity reactions. Drugs are also responsible for renal and cardiac toxicity. Therefore, the treatment of the patients under strict supervision and on indoor basis should be undertaken

Miltefosine

Miltefosine (hexadecylphosphocholine) is an oral drug that was originally studied as an antitumor agent. Subsequent to the serendipitous laboratory finding that miltefosine was active against Leishmania in vitro and, after oral administration in laboratory animals, the drug was developed in a public private partnership for the treatment of visceral leishmaniasis or kala-azar.

The drug is registered with the Drug Controller General of India. The toxicity associated with the drug is minor.

Considering the experience gained in the use of oral drug miltefosine, the Expert Committee, under the Chairmanship of Director General

Health Services, GOI recommended that pilot introduction of this drug for first line treatment should be undertaken in few districts in each of the endemic states. Amphotericin B should be used in cases who do not agree to accept contraception during the treatment with Miltefosine and up to two months after completion of treatment. The patient selection will be on the basis of rK39 dip-stick diagnosis supplemented with splenic aspiration in 5% of the cases.

Subjects for treatment with miltefosine would be patients who confirm the case definition of Kala-azar except pregnant women or any women of child bearing age who does not give an undertaking of refraining from pregnancy (use contraceptive) during the duration of miltefosine therapy and till two months after the end of therapy.

Following would be the inclusion, exclusion and withdrawl criteria

(i) Inclusion criteria:

- a) A clinical diagnosis of active VL or PKDL with consistent signs and symptoms (e.g. fever, splenomegaly, anemia).
- b) Confirmed diagnosis with rK39 dip-stick or with splenic/bone marrow smear examination.
- c) Male or female of ages 2 to 65 years

(ii) Exclusion criteria will be as follows;

- a) Pregnancy or breast-feeding or refusal to use contraceptives during the treatment period and two months after completion of treatment with miltefosine. Miltefosine should not be administered to women of reproductive age group unless they use contraceptives to prevent pregnancy. This should be ensured by providing counseling to patient and her husband. Best contraceptive recommended by the expert group was IUCD as condoms have high failure rate. As an alternative option use of oral pills should be ensured.
- b) HIV positive serology
- c) Infants

(iii)Withdrawal criteria: (during treatment period i.e. to stop the treatment)

- a) Pregnancy

- b) Withdrawal of contraceptive measures

Mode of treatment: The treatment will be provided as a Directly Observed Therapy (DOTS). The patient will be induced to report to the treatment center twice a week for treatment. Experience shows that most patients comply with instructions. The treatment will be provided at all additional PHCs within the districts.

Dosages: After enrollment oral miltefosine treatment will be administered as per following dosage schedule:

- Adults (> 12 years) weighig more than 25 kg: 100 mg miltefosine daily as one capsule (50 mg) in the morning and one capsule in the evening, after meals for 28 days.
- Adults (>12 years): weighing (less than 25 kg) 50 mg, miltefosine daily as one capsule (50 mg) in the morning, after meals for 28 days.
- Children (2-11 years): miltefosine will be given at 2.5 mg/kg daily after meals for 28 days, i.e. 50 mg daily one a day
- The drug is not to be used in the case of children below 2 years of age.

Clinical Response: The response will be judged on clinical grounds, i.e. absence of fever, splenomegaly and anemia.

Adverse Reaction: Adverse reactions to miltefosine are mostly mild. The treating physician should monitor and watch for any adverse reactions. However 98% of the patients are not likely to present with any adverse drug reaction. Even of those who report gastrointestinal reactions, 90% will have vomiting only once a month. Should any skin rashes or gastro-intestinal symptoms develop the doctor may consider stoppage of the drug and refer the patient to higher treatment center. A monitoring of renal and hepatic functions is recommended wherever feasible as about 1% patients may develop nephrotoxicity or hepatotoxicity.

Feasibility of incorporating oral drug Miltefosine as first line drug for treatment of kala-azar cases is known. This drug has already been registered with drug control of India and Phase IV trial has been completed successfully.

...about CDAlert

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