



1. Hospital based surveillance for Kala-azar.

Objective:

The main objective of this Institutional project is to:

- a) monitor changes in disease patterns including therapeutic response and to collect other relevant information,
- b) provide a data base on Kala-azar for researchers to generate and test hypothesis and to carry out clinical and epidemiological research,
- c) provide a regular report to the government and other relevant agencies on kala-azar from a systematic sample of all kala-azar patients attending the hospital,
- d) to develop an early warning system for forecasting an epidemic,
- e) to improve care and introduce better preventive measures.

Progress:

The parasitologically confirmed VL patients, admitted in Indoor ward of this institute, were interviewed through pre-tested questionnaire to collect the information on their demographic, socio-economic status, current and past history of case, besides the therapeutic response based on clinical and laboratory parameters.

Since the inception of this study (Jan. 2001) to March 2007, a total of 1901 (Male 1245, Female 656) were taken into this study. Maximum VL patients were in the age group 5-14 years (39.2%) and male patients (65.4%) were higher as compared to the females (34.6%). About 90% of the patients were from the rural areas of nearby endemic district and 75.2% of the patients hailed from the poor socio-economic strata (Income range Rs. 1000-5000 per month). Nearly 56.4% patients were residing in mud and thatched houses with very poor light condition inside the bedroom and more than 52.1% of the patients kept domestic animals in their houses. Vegetations around the households were reported from 74.5% of the patients.

Clinical and laboratory characteristics were compared between two age groups i.e. ≤ 12 years (Gr. I) and > 12 years (Gr. II). Fever $> 100^{\circ}\text{F}$ with chill and rigor was recorded in nearly 50.2% and 38.0% of cases in Gr. I and II respectively. Splenomegaly (> 5 cm) was recorded in 70.0% of cases in Gr. I and 66.5% in Gr. II, whereas hepatomegaly (> 5 cm) was in 41.1% and 36.1% of cases respectively. Leucopenia was recorded in nearly 74.1% and 51.8%; severe anemia (Hb < 6.5 g/dl) in 37.2% and 26.2%; SGPT within normal range in 84.3% and



72.8%; and SGOT within normal range in 64.2% and 54.2% of cases in Gr. I and II respectively.

Out of various therapeutic options viz. Sodium antimony gluconate (SAG), Pentamidine, Amphotericin B, Amphotericin B lipid complex, Miltefosine and Paromomycin, SAG and Pentamidine had a cure rate of about 58.5% and 66.7% respectively at our Indoor setting and hence, it is no longer in practice currently. Cure rate with Amphotericin B, Miltefosine and Amphotericin B lipid complex were 93.9%, 97.5% and 100% respectively. Under Phase III clinical trial of Paromomycin and Phase II clinical trial of Sitamaquine, initial cure rate observed were 93.6% and 100% respectively.

Table: Regimen wise cure rate

Regimen	Treatment completed	Cured	% cured
SAG	118	69	58.5
Pentamidine	9	6	66.7
Amphotericin B	1424	1338	93.9
Miltefosine	161	157	97.5
Amphotericin B Lipid complex	3	3	100
Paromomycin	110	103	93.6
Sitamaquine	7	7	100

2. A Phase II, multi-centre, open-label, randomised study to evaluate the safety, tolerability and pharmacokinetics of oral sitamaquine compared with amphotericin B in the treatment of visceral leishmaniasis caused by *L. donovani* in endemic areas.

(Sponsor: GlaxoSmithKline; Study No. STQ 105938)

Objectives(s)

Primary objective:

- To characterize the pharmacokinetic profile of multiple doses of sitamaquine with or without food in subjects with visceral leishmaniasis.



Secondary objectives:

- To describe the safety and tolerability of sitamaquine in the treatment of subjects with visceral leishmaniasis, and to compare with amphotericin B.
- To evaluate the efficacy of a 21 day course of treatment with oral sitamaquine.

Progress:

Being one of the clinical center of this multicentric study, 62 patients were screened after obtaining proper written informed consent. After assessment of inclusion and exclusion criteria, only 11 subjects (target 60 subjects including all centers) were randomized for medication (7 in Sitamaquine group and 4 in amphotericin group). The subjects randomized in sitamaquine group (sequence 1-4, categorized as fed and fasting stage) received target dose of 2mg/kg once daily for 21 days and subjects under sequence 5 were treated with intravenous amphotericin B in the dose of 1 mg/kg every alternate days for 30 days. Out of 11 enrolled patients, one patient (amphotericin group) withdrew himself from the study in the mid of the treatment and rest (n=10) completed the treatment. Pharmacokinetics samples were collected as per the time point defined in the protocol. Patient enrollment in this study is over. Follow up of the patients for any AE/SAE, relapse is under way.

Special features:

All the screening, randomization, screening failure, withdrawal, and treatment completion were registered through “Registration and Medication Ordering System (RAMOS)”. The ECG images were transferred to the eResearch Technology through fax for further evaluation. 4-chamber echocardiogram (Colour Doppler) images were captured in DICOM format and transferred through FTP/ CD. Electronic CRF (eCRF) was adopted for entering data of each subject of scheduled time points as well as resolving the queries, raised by Data Management Cell.

Observations:

Initial cure was observed in all the treated subjects. After assessment of clinical biochemistry parameters, it was observed that two subjects (sitamaquine group) developed acute glomerulonephritis like proteinuria at the end of treatment as per the laboratory reports, but it was without any clinical signs and symptoms. In order to further investigate the safety parameters, these two patients were re-hospitalized. It was observed that the elevated protein/creatinine ratio, protein in urine, urine creatinine and albumin in urine reversed to normal



limit without giving any medicine. Data analysis to characterize the pharmacokinetic profile, assessment of safety, tolerability and efficacy of sitamaquine is being done by the Data Management Cell of the sponsor.

3. Susceptibility to Visceral Leishmaniasis (Kala-azar) in human beings - the role of Testosterone.

Objectives:

To evaluate the levels of testosterone in relation to Visceral Leishmaniasis (VL) infection in males.

Progress:

Under this observational pilot study, 20 fresh and parasitologically confirmed VL male cases (age group: 15-45 years) and 20 healthy controls within same age group range were included. After obtaining informed consent, each subject was subjected for estimation of testosterone levels by CIA& RIA technique. Blood samples were collected in fasting stage. Anti-testosterone antibodies were immobilized on microwell plates. This followed further incubation of serum samples from patients and control with HRP labeled testosterone. This was done to allow testosterone in the sample to compete with HRP labeled testosterone for binding to the immobilized antibodies. After washing, enzymes substrate was added and colour development was monitored. It was observed that the amount of testosterone in the samples was inversely proportional to the enzyme activity. The absorbance was measured at 450 nm on ELISA plate reader.

It was observed that testosterone level in control and VL cases with 1+ parasitic load was almost the same, but in VL cases with 5+ parasitic loads it was sharply decreased as compared to control. Preliminary results showed that the level of testosterone in cases and controls do not differ significantly (p -value > 0.05). However, further studies with more number of samples as well as incorporation of cytokine profile are required to arrive at any definite conclusion.

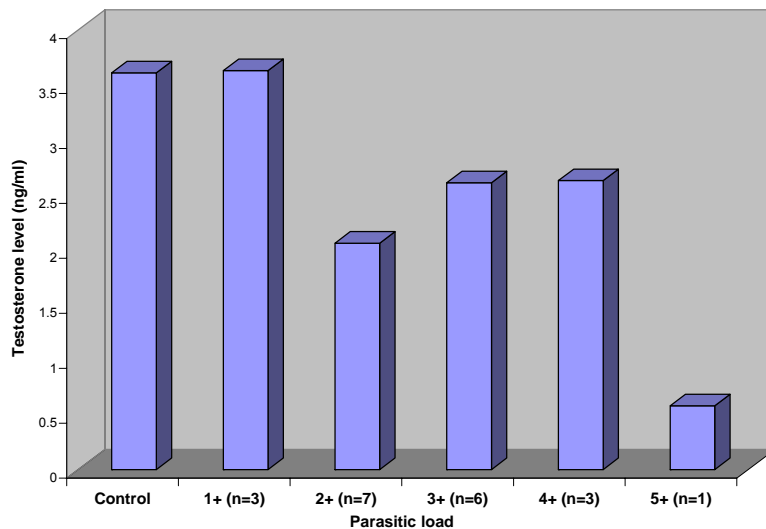


Table: Testosterone level (mean \pm S.D.) in VL patients compared to healthy control

Parameters (ng/ml)	VL cases (n=20)	Control (n=20)	p-value
Testosterone	2.475 \pm 1.8	3.610 \pm 2.55	>0.05

*Normal range: 1.8 – 9.0 ng/ml in male

Figure: Testosterone level in VL cases and its relation to parasitic load



4. PCR based diagnosis of Visceral Leishmaniasis from suspected cases of Kala-azar in Bihar.

Objective:

- To develop a PCR based diagnosis of kala-azar from blood samples and compare the PCR results with conventional diagnostic methods.

Progress:

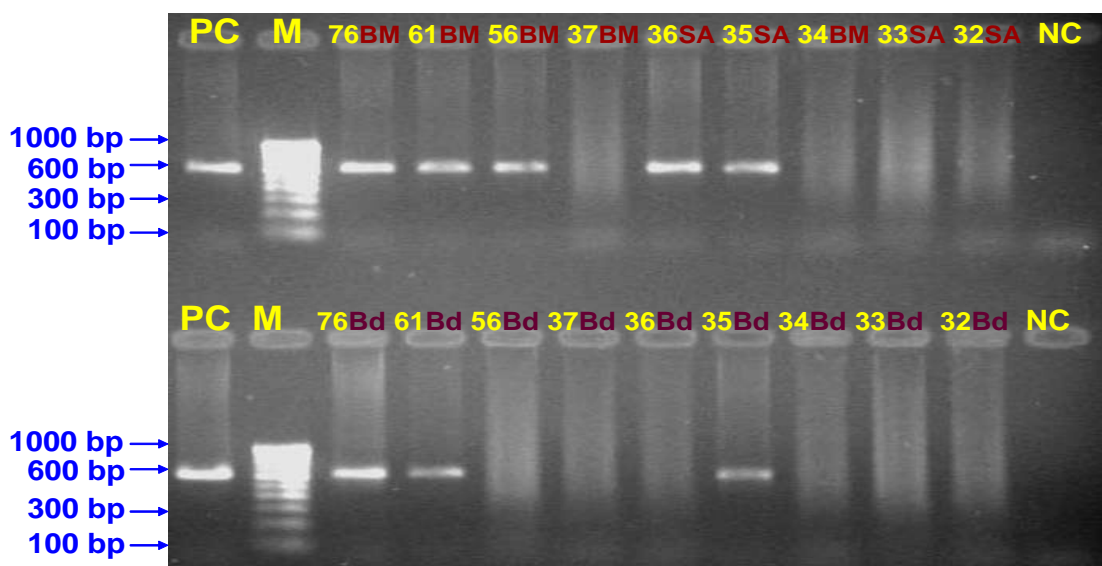
0.2 ml peripheral blood (n=125) and bone marrow (n=25) aspirates samples were collected from suspected kala-azar patients, attending OPD for diagnosis and treatment in the indoor ward of the Institute. Imprinted smear of the aspirates were examined after Giemsa staining for the presence of amastigotes. The aspirates were inoculated in culture medium and incubated at ambient temperature. The wet smears of cultures were examined microscopically for the presence of promastigotes at an interval of 2-3 days up to at least 4 weeks before



considering the samples as negative. DNA was isolated from all the blood and aspirate samples using a QIAamp DNA blood minikit (QIAGEN).

A nested PCR assay to detect parasite DNA in blood and aspirates from suspected kala-azar patients was done. Nested PCR primers encompassing an approximately 600bp fragment internal to the ITS region of rRNA sequence was used. Use of the nested PCR was found helpful from a significant number of samples that were negative in the primary reaction. All samples were tested; results of the test are being under evaluation. Comparisons of the results of the PCR (Blood) with the results of conventional diagnostic methods are under progress.

PCR results of Blood & BM / SA



Lane NC= Negative control, Lanes 32 – 76 =Blood & BM / SA samples,
Lane PC= Positive control, Lane M= Mol. wt. marker.

5. Study of Imprint smear microscopy and PCR application on biopsy from dermal lesions for diagnosis of Post kala azar dermal leishmaniasis cases in Bihar.

Objectives:

- To apply the PCR for diagnosis of PKDL and compare it with the conventional microscopy of skin biopsy imprint smear.
- PCR application in PKDL cases after treatment and during follow up.



Progress:

PKDL cases (n=38) were selected from RMRIMS outdoor and nearby endemic villages of Bihar. They were asked about any past history of Kala-azar, treatment and duration of skin lesions and examined for the type of the lesions (i.e. macular, papulo-nodular and erythematous), site on the body, coalescence and any loss of sensation.

Biopsies were collected aseptically from different skin lesions of the PKDL cases after obtaining informed consent from the patients. Imprint smears on clean glass slides were prepared and stained with Leishman /Giemsa stain for detection of leishmania parasites under microscope. Blood sample were collected for haematological tests. Biopsy samples were collected in the Tris buffer solution for PCR study. PCR was carried out to detect the sequence specific to whole ITS region of the ribosomal RNA (rRNA) gene of *L. donovani*.

Tissues were grinded in Tris EDTA buffer solution (pH 8.0); washed twice with T.E. buffer and DNA was isolated by commercially available 'Qiagen' kit. The amplified PCR products reactions were visualized on 1.5 % agarose gel, using a DNA marker. Samples were scored as positive when a PCR product of 600 bp could be detected as in the positive controls. The results obtained by PCR were compared with microscopy of the imprint smear. Six biopsies from cases with fungal skin lesions and leprosy were used as negative controls for PCR test, whereas leishmania parasites culture isolates from PKDL cases were used as positive control. Out of 38 PKDL cases, 19 had only hypopigmented macular lesions and the other 19 cases had mixed lesions of papulonodular-erythematous with or without macular lesions. Past history of kala azar was present in all the cases with duration of 1 to 16 years.

In imprint smear microscopy, parasite positivity was found in 17 (89.47%) papulonodular cases and only in 7 (36.8%) macular cases, with presence of mononuclear cells, histiocytes and lymphocytes in the smear. The biopsy smear from skin lesions of fungal infection and leprosy cases were observed negative for the Leishmania parasite.

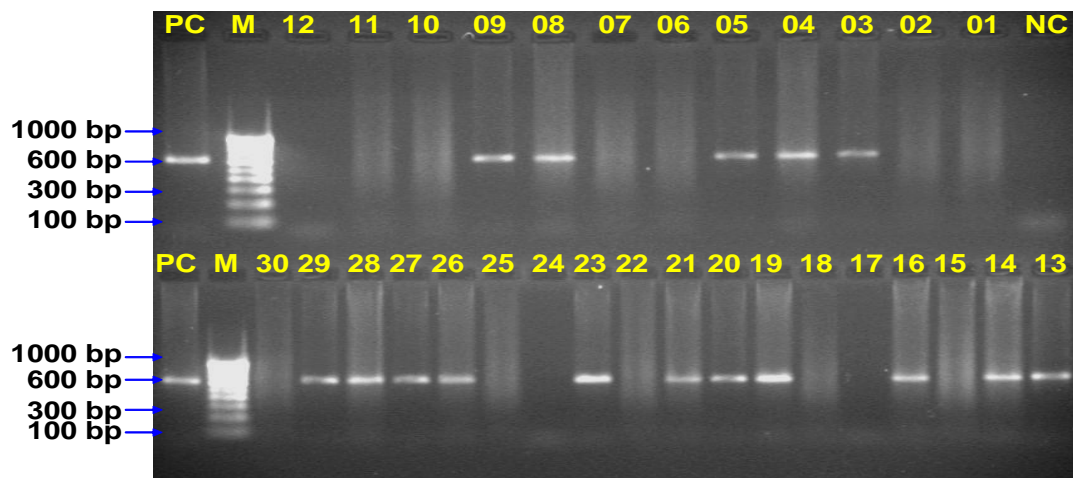
PCR showed positivity in 17 (89.47%) papulonodular cases of PKDL. PCR positivity was significantly higher in the hypopigmented macular cases of PKDL, where it was positive in 16 (84.21%) cases, however the microscopy could detect only 36.8% positivity in the same group. Both PCR and imprint smear were negative in the negative control subjects. The study is still in progress to include 50 fresh cases of PKDL for final interpretation.



Table : Compare of microscopy and PCR for Leishmania positivity in different types of lesions of PKDL cases.

PKDL Lesions	N	Microscopy of Biopsy Imprint smear	PCR of Skin Biopsy
Papulo-nodular lesions	19	17 (89.47%)	17 (89.47%)
Hypopigmented macular lesions	19	7 (36.8%)	16 (84.21%)
Total PKDL cases	38	24 (63.15%)	33 (86.84%)
Negative controls	6	0	0

Nested PCR of skin biopsy samples



Lane NC= Negative Control, Lanes PL-01-30= Skin biopsy samples, Lane PC=Positive Control, Lane M= Mol.wt. Marker.

6. Establishment of national repository for Leishmania parasites and sera bank.

Objectives:

1. To isolate and maintain Leishmania parasites from different clinical materials as well as from the vector of Kala-azar, *P. argentipes*.



2. To cryopreserve different isolates of *Leishmania* of different geographical areas and WHO referral centers.
3. To characterize the various isolates of *Leishmania*.
4. To preserve sera samples of Kala-azar and PKDL cases; other diseases; and healthy controls.

Progress:

Leishmania parasites from splenic/ bone marrow aspirates and dermal lesions of kala-azar and PKDL cases respectively, hailed from different endemic areas, have been isolated and maintained *in vitro* in biphasic media. Out of 30 cryopreserved isolates, 8 were revived and 20 new isolates (Kala-azar 19; PKDL 1) are being maintained for its cryopreservation in liquid nitrogen at -20°C. The Amphotericin B unresponsive (N=2) and PKDL (N=1) isolates have been adapted in culture for inoculation in Balb/c mice for *in-vivo* maintenance. The study is in progress.

7. Molecular characterization of SAG responsive and unresponsive kala-azar isolates of Bihar.

Objective:

- To demonstrate, if any variation exists in SAG responsive & unresponsive isolates of kala-azar cases of Bihar using molecular tools.

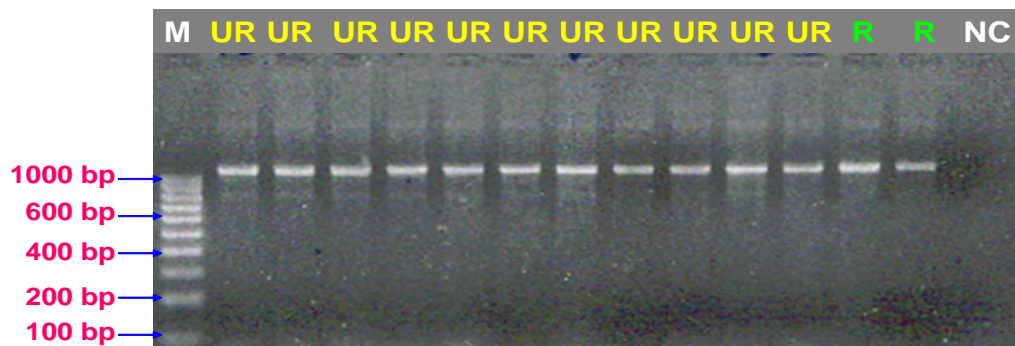
Progress:

After primary isolation & culture adaptation, mass cultures of different clinical isolates of SAG responsive (n=2) & unresponsive (n=10) strains were carried out in monophasic media. DNA was isolated from these isolates by chemical method (i.e. proteinase K, SDS and CTAB/NaCl) and ITS region of the rRNA gene was amplified from all isolates. PCR products were analyzed in 1.5% agarose gel and a band of 1100bp (approx.) was found in all. In previously experiments four restriction endonucleases i.e. *Hha* I, *Rsa* I, *Hae* III and *Taq* I, were used, out of which only *Taq* I demonstrated the differentiation in banding patterns among the SAG (R) and SAG (UR) isolates. This time amplicons were digested with *Mae* II (*Tai* I), *Hpy* F10VI (*Mwo* I), *Tru*1 I (*Mse* I), and *Tas* I (*TspE* I) restriction endonucleases. PCR–RFLP patterns showed 3 restriction cutting sites (i.e. 140, 250, & 561bp) with *Mae* II



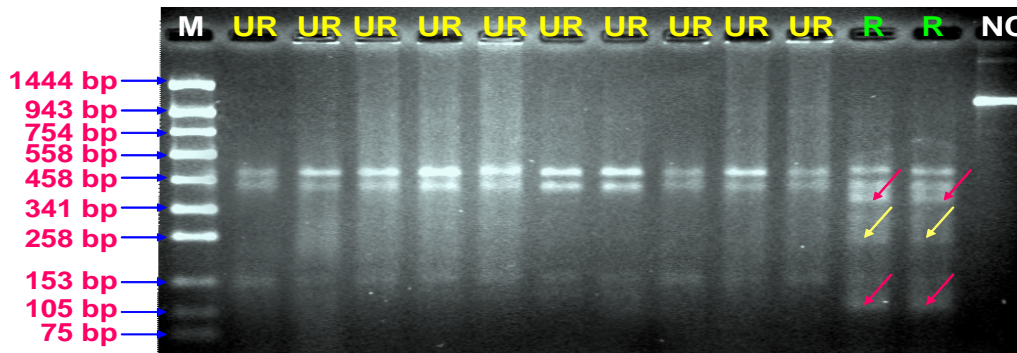
(*Tai* I), 2 restriction cutting sites (i.e. 360 & 647bp) with *Hpy* F10VI (*Mwo* I) and 2 restriction cutting sites (i.e. 769 & 820bp) with *Tru*1 I (*Mse* I) in all (SAG-responsive/unresponsive) isolates. But differences were observed among the SAG (R) and SAG (UR) isolates restricted with *Tas* I (*TspE* I) restriction endonucleases. A band of 400bp (approx.) was observed in SAG (R) isolates but not in SAG (UR) isolates restricted with *Tas* I (*TspE* I).

Amplification of ITS region



Lane NC =Negative control, Lanes R =Responsive isolates (SAG),
Lanes UR =Un responsive isolates (SAG), Lane M =Mol. wt. marker.

Result of PCR—RFLP *Tsp* EI (*Tas* I)



Lane NC =Un digested, Lanes R =Responsive isolates (SAG),
Lanes UR =Un Responsive isolates (SAG), Lane M =Mol. wt. marker.

8. Malnutrition as a risk factor in the severity of visceral leishmaniasis.

Objectives:

- To identify and assess the nutritional markers/factors in the malnourished VL patients
- To evaluate the correlation between malnutrition factors and VL

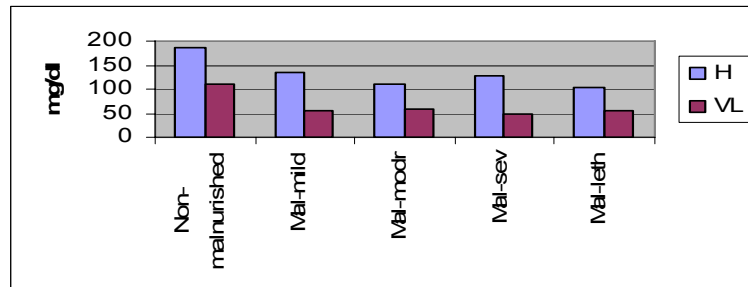


- To assess the nutritional factors predisposing to severity in VL.

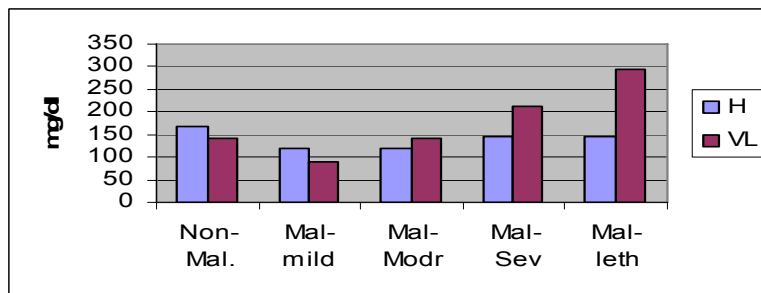
Progress:

Malnourished VL subjects were categorized into different sub-groups, i.e. mild, moderate and severe according to their Body Mass Index (BMI) as well as by clinical assessment. Various nutritional biochemical laboratory tests for malnutrition based on BMI and their correlation with the severity of the disease were studied. The nutritional parameters studied were albumin, Cholesterol, Triglyceride, LDL, HDL, apolipoproteins, copper, zinc, magnesium and iron. Severe down regulation of Cholesterol was observed in all the malnourished VL cases, which suggest that leishmania parasite might be playing an important role in the down-regulation of cholesterol in order to establish the infection. Hypertriglyceridemia and down regulation of Iron and Zinc were the other parameters observed in VL malnourished patients. The study is in progress.

Nutritional Marker(CHOLEST.)



Triglyceride



9. Role of CD2 Antigen in T-cell signal Transduction pathway in Visceral Leishmaniasis



Objectives:

- To understand the role of CD2 deficiency in VL and its consequences on CD4 subpopulation of T-cells
- To find out the possible means for modulation of this pathway as a mechanism to ensure protective cytokines in patients.

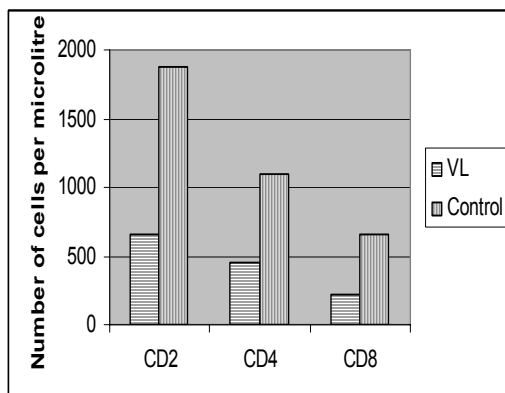
Progress:

The VL patients were observed with less CD2 expressed on the cell surface of T-lymphocytes and human CD2 optimized T-cell response to proliferate in response to *Leishmania* antigen. Further, it has been observed that the T-cells of VL patients reverted the gross impairment in the Protein Kinase-C mediated signaling in the presence of anti CD2 antibodies. The above changes also enabled T-cells to alter their behavior for cytokine production. The major impact noticed was on CD4 lymphocytes blast gate population, which accounted for the majority of the Interferon-gamma (IFN- γ) producing cells after anti-CD2 triggering. In a separate experimental, it was demonstrated that activation of CD2 antigen by anti-CD2 antibody uplifts the Th1 associated CD4 cell response as IFN- γ production by T-cells occurred even in absence of APCs. CD2 pathway of T-cell activation was further studied in terms of its impact on Th2 associated CD4 response. It was observed that CD2 on T-cells once activated it downregulates IL-4 production, which remained unaltered even though recombinant IL-4 was added to promote CD4 cell differentiation into Th2 during culture. Thus a critical role of CD2 in transducing a negative signal for IL-4 is identified in Visceral Leishmaniasis, which once decreases, brings a shift in Interferon-gamma production.

Figure 1: Immunophenotyping of CD2, CD4 and CD8 + T-cells in VL patients and control.

A.

B.



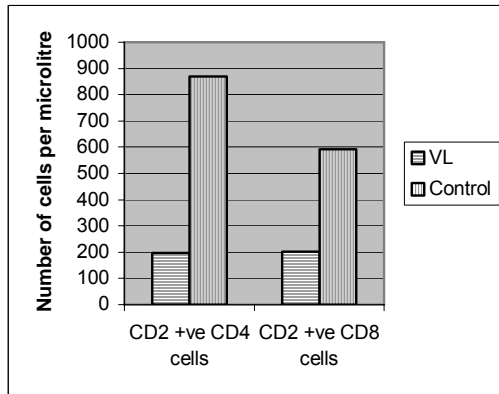
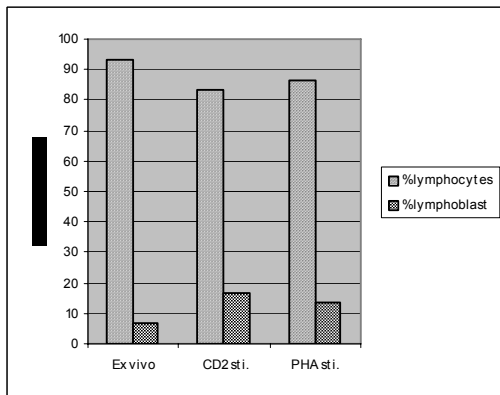


Figure 2: Flow cytometry analysis on reversal of T-cell activation pattern in response to *L. donovani* antigen upon activation of CD2 antigen in VL patients.

A.



B.

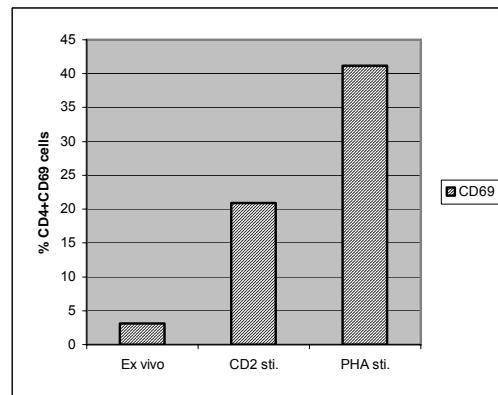
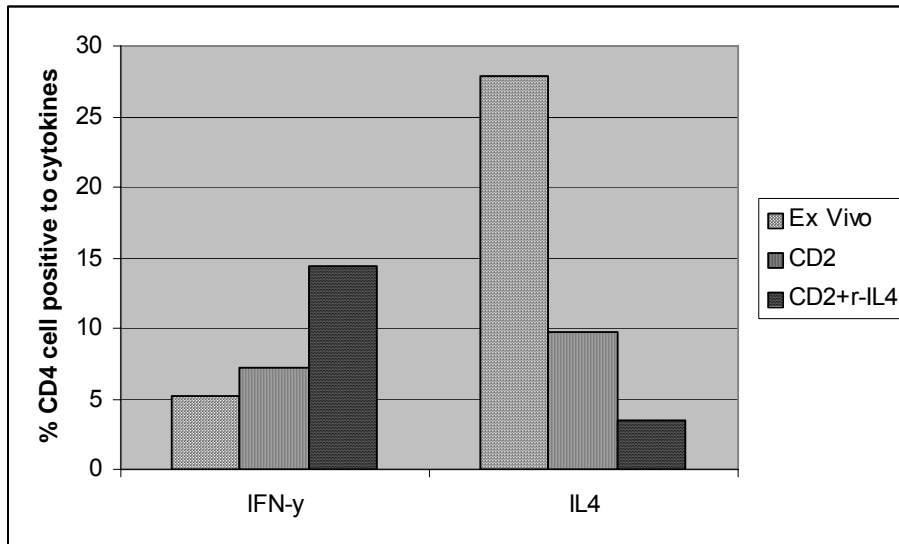


Figure 3: CD2 activation in VL patients influences IFN- γ responsiveness in T-lymphocytes by down regulating IL4 production.



10. Role of proteophosphoglycan in protection of Visceral leishmaniasis.

Objectives:

- To isolate, purify and characterize proteins of *L. donovani* promastigotes with and without Glyco-phospholipid anchors.
- To assess whether *L. donovani* surface proteins with and without GPI-anchor may be potential targets for anti-host cellular immune response, such as TGF- β and Interleukin-10.
- To assess a correlation of *L. donovani* proteins with and without GPI-anchor with multi-drug resistance {ATP-binding cassette (ABC) transporters- *pgp-1*}.

Progress:

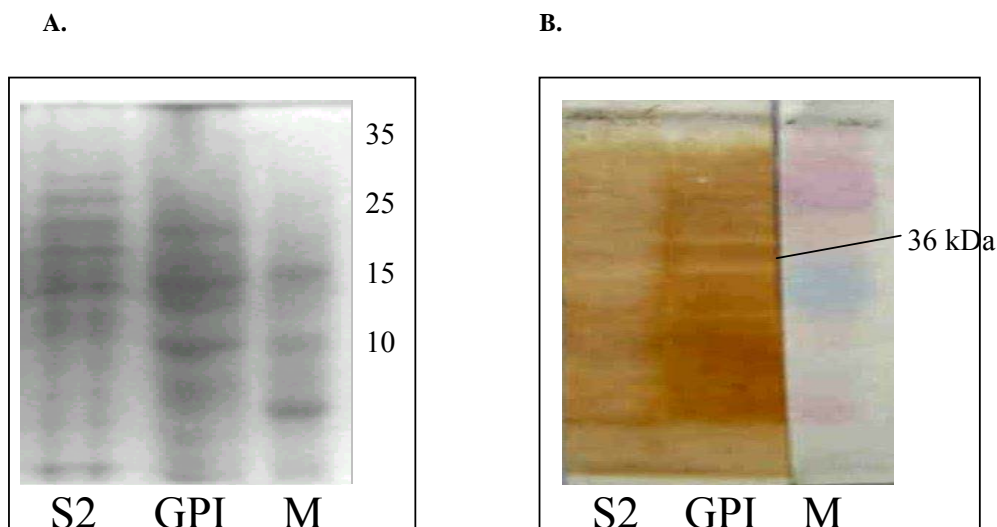
We investigated the prospect of Glycoinositolphospholipid (GPI)-anchors on proteins of *L. donovani* promastigotes as a potential target of cellular immunity particularly those that govern regulatory mechanism to add in therapeutic unresponsiveness in VL patients. *L. donovani* promastigotes (10^8) were equilibrated with 2 ml TBS and 2ml Triton-X 114. Following incubation, suspension was pelleted and supernatant suspended in ice-cold PBS was subject to 37°C water bath. The centrifuged material containing soluble proteins (upper phase) and trans-membrane protein anchored by Glyco-phospholipid structure (lower phase) were collected. NCP-blotted Polypeptides of these proteins were probed with mouse anti-human *pgp-1* and MRP-1 and on the basis of reactive bands in a western blot, relevance of



these proteins with drug resistance was established. In a separate experimental set-up, Ficoll separated PBMC (2×10^6 ml) suspension from VL patients (n=5) were re-stimulated in 96 well round bottom micro-titre plate in presence and absence of these proteins for 48-72 hrs. The TGF- β and IL-10 level in cells in the presence or absence of the *Leishmania* proteins were measured using cytokine based ELISA.

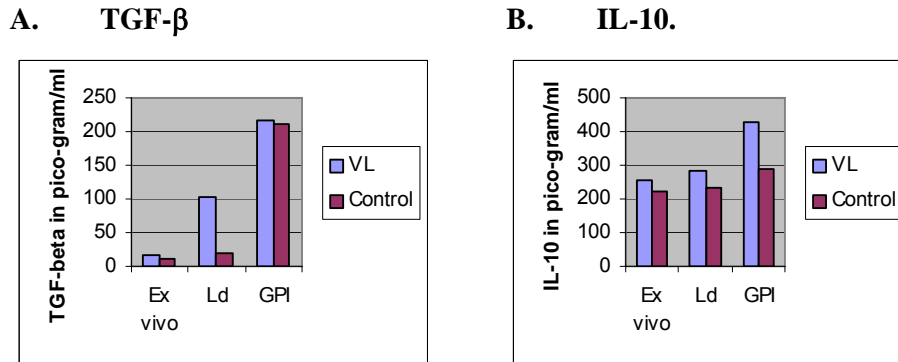
Both GPI-anchored and GPI-non-anchored surface proteins were shown to contain polypeptide fraction that phosphorylated significantly the pgp-1. The 36kDa protein with and without GPI-anchor significantly phosphorylated pgp-1 reactivity. Efficient replication of *Leishmania* parasite is initiated with cellular immune activation of Th2 (IL-10 and IL-4) associated CD4 cell response during which possibly the protective immunity (Th1) is abrogated by Th2+CD4cells with TGF- β support. Thus, we next examined whether these *Leishmania* associated proteins were associated with activation of anti-host cellular response to facilitate *Leishmania* replication and promote resistance in patients. To address this, we used antigen-induced analyses of the induction of TGF- β and IL-10 secretion. VL patients produced a 5-fold more TGF- β compared to control. An increase in induction of TGF- β of 2.10-fold and 2.12-fold in response to GPI-anchored *Leishmania* protein and PHA, respectively, was noted in Patients. Overall, though there was no significant difference in the induction of IL-10 ex-vivo, after LD and GPI-protein induced stimulation in patients; we observed that there was significant increase in IL-10 by cells stimulated with GPI-proteins compared to control. Further studies are in progress.

Figure 1: A. Antigenic profile soluble protein (S2) and GPI anchored protein. B. Immunoblotting of the antigen with anti pgp-1 antigen.





**Figure 2: A. *Leishmania donovani*: GPI-anchored protein activates TGF- β
B. IL-10 associated cellular mechanism during VL infection.**



11. In vitro, role of antigen of *Leishmania* isolates of SAG responder and non-responder patients in IFN- γ & IL-4 production by similar sets of T-cells.

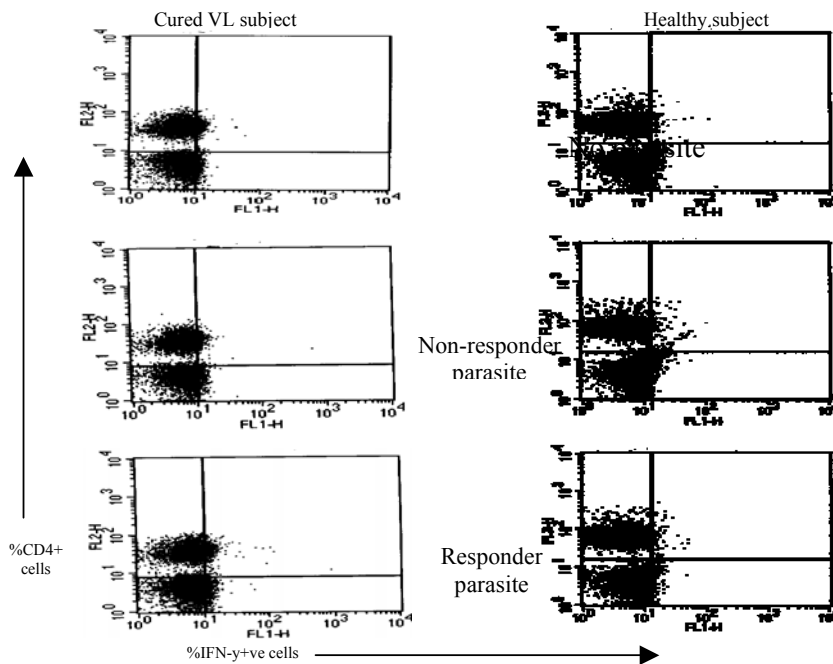
Objective:

To evaluate cytokines (IFN- γ & IL-4) production in two similar sets of T-cells collected from visceral leishmaniasis cured subjects and stimulated with whole antigen of SAG responder and non-responder isolates of *L.donovani*.

Progress:

The mononuclear cells from collected samples were stimulated with whole antigen of SAG responder and non-responder isolates and stained with anti-human CD4PE monoclonal antibodies. The cell accumulated IFN- γ was detected with anti-human IFN- γ labeled FITC monoclonal antibodies. The images of six samples were acquired by Flow Cytometer and data were analyzed by BD cell quest software. The work on IL-4 detection in T-cells of VL cured subjects stimulated with whole antigen of SAG responder and non-responder is in progress.

The data of IFN- γ production suggested approximately two fold increased production of IFN- γ in responder than non-responder parasites against T-cell of cured patients. Further work is under progress.



12. Study on Immunopathology of Post Kala azar Dermal Leishmaniasis (PKDL): T-cell subsets.

Objective:

To observe the changes in T cell subsets in PKDL lesions and in circulation in relation to VL cases and to understand its role in the Pathogenesis of PKDL

Specified Objectives:

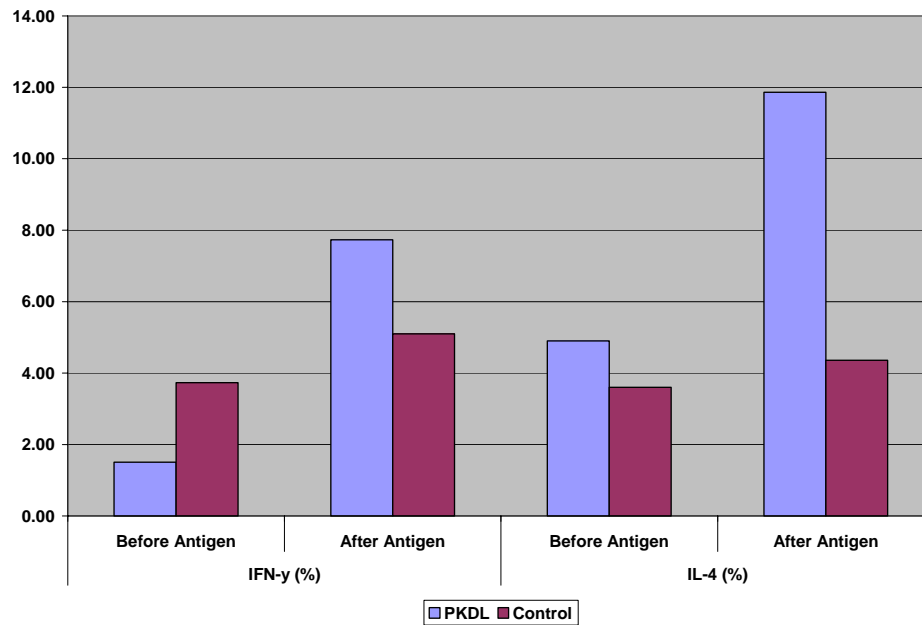
- To determine the level of T helper and T suppressor cell in skin lesions and in the peripheral circulation of PKDL cases..
- To measure the levels of cytokine (IL-2, IFN- γ , IL-4 and IL-10) in PKDL cases and compare it with VL and control subjects.

Progress:

The Cytokine study has been conducted in 6 PKDL cases and 5 normal controls. It was found that cytokine response pattern in PBMNC of PKDL cases does not show much difference from kala azar cases as there is downregulation in IFN- γ producing abilities of CD-4 cells with an almost 2-fold rise in the frequency of CD-4 cells positive for IL-4.



Intracellular IFN- γ and IL4 production by CD4 cells in PKDL cases compared to control



13. Crucial role of plants' extract in propagation of *Leishmania donovani* promastigotes.

Objective:

- To explore the possibilities of some plants' extract
 - as a source for replacement of blood/blood products/FCS/serum in routine culture of *L. donovani* promastigotes.
 - as a source of antileishmanial compound, if show lethal effect.

Progress:

Based on initial suggestive observations, fresh lot of plants' extract of some plants' (n=13) that belong to different families (n=10) having different characteristics such as habit, habitat, occurrence, flowering season, duration of plants etc. were prepared in distilled water. Three different culture media (2 commercially available i.e. RPMI-1640, pH 7.4 and Schneider's insect medium, pH 7.2; and another one LGPY, pH 7.4) were supplemented with 20% plant's extract, sterilized by Millipore filter. The plain medium was taken as negative control and medium with 10% FCS was taken as positive control. Equal numbers of washed log phase promastigotes were inoculated in experimental as well as control medium and incubated at 24°C, cultures were sub-passaged in the respective fresh medium at every 5th days. Non-existence of alive cell / lethal effect on promastigotes was confirmed by sub-culture in LGPY



medium having 10% FCS. In RPMI-1640, lethal effect of 2 plants' extract was also observed even after addition of 10% FCS. Adequate proliferation was exhibited by 9 plants' extract as supplement in LGPY medium for 74 sub-passages, which is quite enough to ensure its use for long term maintenance of promastigotes.

To find out more choice in selection for getting plants' extract easily & economically in different seasons and places, 19 new plants' extract were also screened in LGPY medium, out of which 8 exhibited adequate proliferations of promastigotes in continuous sub passaging over 24 successive sub- passages. One plant's extract showed lethal effect on promastigotes in LGPY medium, and in RPMI-1640 even after addition of 10% FCS. (Table 1).

Out of total 21 plants' extract, 2 exhibited thermal stability as they supported promastigotes proliferation in long-term successive sub passaging over 22 sub passages when used as supplement after autoclaving at 121°C for 20 minutes. Plants' extract kept at -20°C for 3 years and at 4°C for 1 year exhibited luxuriant growth indicating long storage stability/ self life. Three different plants' extract (20%) were supplemented to 1% agar base in LGPY medium and inoculated with 3 cells/ plate. Whitish mucoid colonies (size 3-4 mm approx.) were observed on 10th day in each plate indicating the capability of these plants' extract to promote cell growth from single cell.

As a pilot experiment to demonstrate whether plant extract can be used in primary isolation of *L. donovani*, LGPY medium, supplemented with FCS (10 & 20%) and different plants' extract (n=6), were inoculated with splenic/bone-marrow aspirate. Except one, all the five plants' extract supported primary isolation of parasite within 4-5 days. More sets of experiments is in process to authenticate the observations.

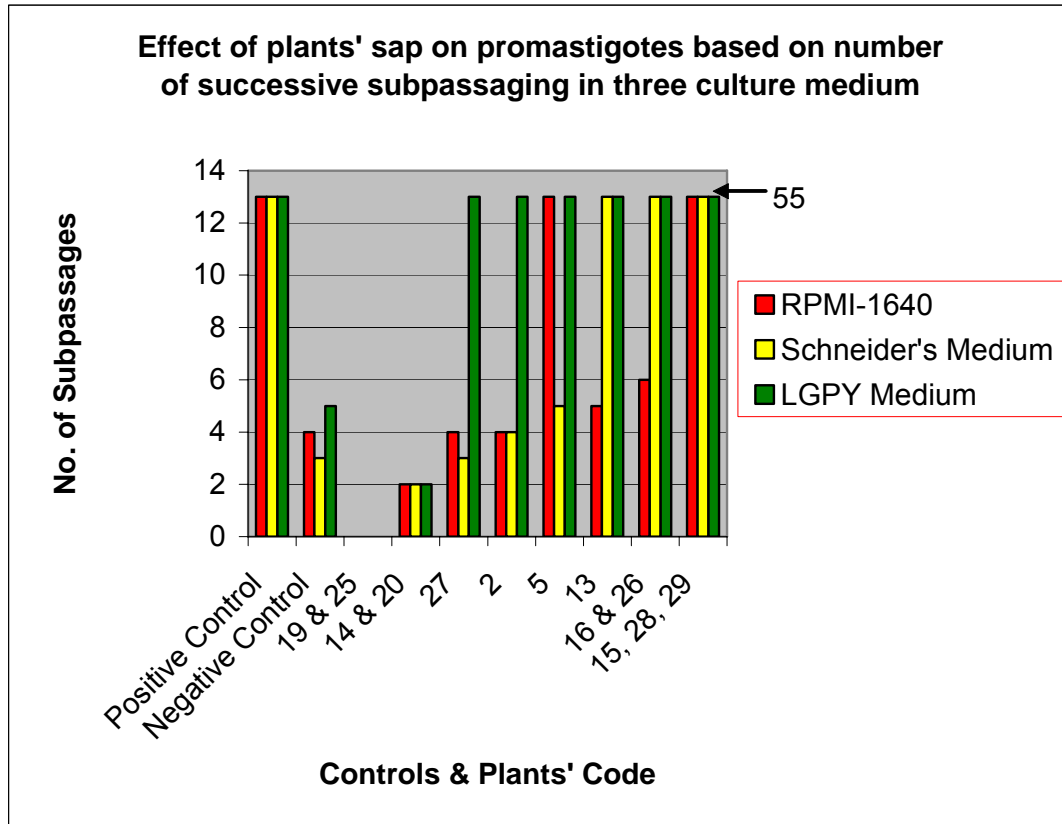


Table 1: Effect of plants' extract on promastigotes based on number of successive sub-passages.

Effect on promastigotes	Description of effect	Survival in No. of sub-passages	PE code/Total No.
Lethal	Non-motile / deformed cells	0	32 /1
Harmful	Cells sustainment < control	1 to 4	33, 34, 36, 45, 46, 49/6
Short-term survival	Cells sustainment = control	5	35, 37, 38, 39 /4
Long-term survival	Cells sustainment > control (continued)	24 cont.	31, 40, 41, 42, 43, 44, 47, 48 /8



Positive control	Cells sustainment > control (continued)	24cont.	
Negative control		5	

14. Identification of sibling species of *Phlebotomus argentipes* population in Bihar

Objectives:

- To identify the sibling species of *Phlebotomus argentipes* in Bihar
- To find out the relevance of sibling species vis-à-vis Kala-azar endemicity

Progress:

The work was initiated with the scrutiny of different forms of *P. argentipes* based on relative length of ascoid and 4th antennal segment. The sandflies were collected from Gulmehiyabagh village of Patna district. Individual sandflies were confined separately in rearing pot. After egg laying the dead sandflies were mounted in Hoyer's medium and the head was observed minutely under microscope for the measurement of ascoids and 4th antennal segment and based on the observation, all pots were marked as 0.2, 0.3, 0.4, 0.5 and 0.6. For each type a bigger rearing pot was taken and approximately 100 eggs of each type were collected and observed for further developmental stages like all four instars of larvae and pupae, morphologically.

No significance difference was observed during comparison of different developmental stages. The adults (male and females) of each type were separated just after emergence and cross-mated among different forms for reproductive isolation. No mating was observed through naked eye during 12 hours of close observation from emergence. The study is under progress for more set of experiments. Isoenzyme and molecular assay will be followed to find out the genetic variability in different forms of *P. argentipes* from different geographical regions.

15. Exploratory investigations to detect the existence of chemical communication between male and female *Phlebotomus argentipes*



(Old-world Sandfly), Indian vector of Kala-azar for mate and host location. (Sponsor: DBT)

Objectives:

- To develop improved laboratory culturing methods for larger scale fly output of *P. argentipes*.
- To design simple laboratory experiments to detect the existence of semiochemical mediated communication for behavioral responses between male & female *P. argentipes*.
- To isolate semiochemicals released by male by *P. argentipes* by solvent extraction or by volatile entrainment technique.
- To study behavioral aspects for validation of the chemical communication between male and female *P. argentipes* using male extracts in Y-tube olfactometer.

Specific objectives:

- To establish the critical parameters for improving *P. argentipes* colony with increased fly output
- To assess experimental evidence for the role of chemical communication in *P. argentipes* for host and mate location.
- To setup cross mating experiments to establish species homogeneity in *P. argentipes*.

Progress:

Wild sandflies were collected from highly endemic Muzaffarpur and Vaishali districts for establishment of colony in the insectarium for this study. So far, 65 sandflies were collected in two field visits. Out of 65 sandflies, 23 were fed and were confined for egg laying. The fly out put from these confinements was 160. Further improvement in sandfly rearing on larger scale is under progress.

Table 1: Collection of sandflies from endemic districts

Date	Name of the District	No. of sandflies collected			Total	Temp. ° C	RH %
		No. of ♂	No. of ♀				
			Fed	Un fed			
09.02.07	Muzaffarpur	20	9	6	35	16	95
14.02.07	Vaishali	10	14	6	30	19	100



Table 2: Number sandflies emerged from wild caught flies

Date of confinement	No. of ♀ confined	Total no. of eggs laid	No. of eggs/female	No. of eggs hatched	% hatching	No. of adults emerged	
						♂	♀
09.02.07	9	100	11	70	70	33	37
14.02.07	14	130	9	104	80	48	42

16. Control of Indian Kala-azar by genetic changing of the symbiotic bacteria of the vector, *Phlebotomus argentipes*.

Objectives:

- To identify the symbiotic bacteria from the gut of *Phlebotomus argentipes*
- To transform the bacteria genetically and ensure the paratransgenic transmission of GM bacteria
- To ensure development/nondevelopment of *Leishmania donovani* in the presence of modified bacteria

Progress:

In the previous year, 20 morphologically different bacterial colonies were identified from the gut of *P. argentipes* collected from different endemic villages of Bihar like Wajitpur Majhauri (Muzaffarpur), Mahnar (Vaishali) and Mohanpur (Samastipur). The identified colonies were mostly gramnegative *Pseudomonas*. To assess the symbiotic association of all colonies with *L. donovani* viability test was conducted with metabolites of all colonies which were serially diluted in RPMI with 20% FCS in 96 well plate. The wells were charged with promatigotes (2×10^5 / ml) and incubated for 3 days at 25⁰C. The viability was tested under haemocytometer resulted good growth with one metabolites than control. Further work of paratransgenic bacteria is under progress.

17. Vector Biology in Control trial (KALANET Project; European Commission)

**Objective:**

- To demonstrate whether the blanket use of LLINs in a community provides any mass effect, which would provide protection to those in the community who fail to use LLIN for any reason, i.e. due to a reduction in the Entomological Inoculation Rate (EIR) by
 - reduction in sandfly abundance and/or
 - reduction in the sandfly infection prevalence.

Both parameters were estimated monthly in 3 villages with LLIN and 3 villages without LLIN.

Progress:

The efficacy of long-lasting impregnated bednets (LLIN) on VL prevention is being assessed as a community based intervention trial in Bihar. The study aims to demonstrate a 50% reduction in *L.donovani* incidence rates in the intervention group compared to control. Six clusters of households were randomly allocated to the intervention and the control arm. Complementary to the trial, a study to monitor the entomological impact of the intervention is being conducted.

In each cluster 25 households were given temporary number (T1 to T25) after search for vector by two insect collectors for 10 minutes in up to two rooms. The houses positive for vector were selected and recorded during the demographic survey. Out of 25 households, 10 households and 10 cattle sheds (CS) in 6 clusters were selected randomly and followed for one night per month from September to November 2006.

In every month, light traps were set up in 10 selected households and untreated bed nets were provided to the household members for use. Sandflies were collected by light trap method. After light traps collection, the treated bed nets were returned and aspiration was done in two rooms per house and cattle sheds. The collected sandflies and mosquitoes were preserved for further study.

Installation of CDC light trap for collection of sandflies



18. Efficacy, acceptability and cost-effectiveness of long lasting insecticidal nets in the prevention of Kala-azar (KALANET: European Commission)

Objectives:

- To evaluate the efficacy of long lasting insecticidal bednets (LLIN) in the prevention of VL in the endemic focus of Nepal and India
- To estimate the cost and cost effectiveness
- To examine the acceptability of LLIN
- To evaluate the efficacy of alternative control technology

Specific Objectives:

Under Work Package (WP)-7, the specific objectives are as follows:

- To measure and compare efficacy of two types of Long lasting insecticidal bed nets
- To measure and compare efficacy of other community based vector control intervention methods

Progress:

In the previous pilot study to evaluate the efficacy of different collection methods of sandflies in Indian context, various indoor collection methods viz. flash light and aspirator; sticky



traps; CDC-light traps and CDC-VU traps were adopted. Flash light and aspirator were used for collection from resting walls in the morning where as sticky traps, CDC-light traps and CDC-VU traps for moving sandflies over night. CDC light trap was found more effective in collecting moving sandflies during night.

During first year of the study, three types of bed nets were used namely a) OLYSET: wide mesh (4mm x 4mm), polyethylene, blended with permethrin 2%; b) PermaNet 2.0 (second generation): with small mesh (156 holes/inch²) and c) Traditional net. A five-arm study including Permanet, Permanet control, Olyset, Olyset control and Traditional untreated nets, was set up in three highly endemic hamlets of Gulmehiya bagh (Patna, district), Rasoolpur and majlishpur (Vaishali, district).

Per hamlet 10 mix and 10 normal houses were selected for their high vector density. Labeled nets were distributed during the week after the first survey. The entomological efficacy was estimated on sandfly abundance during one sandfly season (April-June 2006). Collection of sandflies was conducted using CDC- light trap during night time followed by morning resting collection with aspirator before the intervention (week 0), 3, 6 and 9 weeks after treatment. The bed nets were utilized properly under constant supervision. The data is under compilation.

Impregnated bednets in the study area



- 19. Cost Effective Integrated Vector Management as a Contribution to the Visceral Leishmaniasis Elimination Initiative in the Indian Sub-continent: a multi-centre study. (Sponsor: WHO/TDR)**

**Objective:**

- To contribute evidence for more effective vector management in support of the VL elimination initiative.

Specific objectives:

- To evaluate the safety and effectiveness of IRS/ITN/Eco-environmental management in reducing vector density & transmission of VL.
- To evaluate cost of IRS/ITN/eco-environmental management.
- To identify most cost-effective intervention amongst IRS/ITM/eco-environmental management.
- To evaluate acceptability of the three strategies.

Progress:

Six hamlets were selected based on high incidence of VL cases in Vaishali district of Bihar for IRS and Muzaffarpur district for LLIN. Altogether 24 clusters were selected for the study. In each hamlet, based upon the types of interventions, clusters were selected for comparing 3 interventions namely IRS, LLINs, Eco-environmental and one cluster was selected as control. From each clusters, 5 households were randomly selected after stratification into mixed houses (N=2) or human houses (N = 3).

Baseline estimates: Vector density was measured in the month of November and December and the relative density of the sandflies were estimated for each house with 2 overnight CDC light traps collections. Based upon the average vector density, 6 groups of 4 clusters/villages were identified. The clusters in each group were listed 1 to 4 and randomly allocated for interventions.

Arm A LLINs

In every month, light traps were set up in 5 selected houses before and after intervention. Untreated bed nets were provided to the household members for use and sandflies were collected by light trap in night. After light trap collection, the treated bed nets were provided and aspiration was done in two rooms per house and in the cattle sheds. The collected sandflies and mosquitoes were preserved for further study.

Arm B IRS



IRS was carried out in coordination with district public health centre (PHC) as per Government of India (GOI) policy. Prior to spraying, the head of households were informed of the procedure and date of DDT spray and asked to sign a consent form. An entomological technician was deputed for the insecticidal spray, particularly in targeting the cracks and crevices in the households and the peridomestic sites. The work is in progress.

Bioassay test to ascertain the efficacy of LLIN



Preparation of DDT solution for IRS



20. Evaluation of impact of DDT indoor residual spraying being used in Kala-azar control programme on the disease prevalence.

Objective:

- To assess the susceptibility of *P.argentipes* against DDT.

Progress:

DDT Sensitivity test reveals the development of tolerance in *P. argentipes* in all the four districts i.e. Bochaha (Muzaffarpur District), Patepur (Vaishali district), Bande (Samastipur district) and Runisaidpur (Sitamarhi district). However *P. argentipes* of Bahapur (Patna district), which has no history of spraying since last ten years, showed 100% sensitivity against 4% DDT.

In order to study the epidemiological impact of DDT and vector resurgence, an endemic village, Belladam (Patepur PHC) in Vaishali district, was selected having maximum number of cases (8 cases) in the year 2004–05. The population of this village was 1448 in 221 households. DDT spraying was carried out under our supervision and all parameters like pre



and post evaluation of vector density, bioassay test was carried out. Simultaneously, pre post sand fly density was monitored in 11 districts the result in the following tables.

Table 1: Pre spray vector density observation

District	Date	Present status of sandfly		Total sandflies collected	MHD
Muzaffarpur, PHC- Bochaha	07.04.06	<i>P.argentipes</i>	63	79	13.1
		<i>P.papatasi</i>	06		
		<i>Sergentomyia spp</i>	10		
Sithamarhi, PHC-Runisaidpur	18.04.06	<i>P.argentipes</i>	48	62	10.3
		<i>P.papatasi</i>	14		
		<i>Sergentomyia spp</i>	00		
Nalanda, PHC- Chandi	19.04.06	<i>P.argentipes</i>	60	66	8.25
		<i>P.papatasi</i>	02		
		<i>Sergentomyia spp</i>	04		
Samastipur, PHC- Tajpur	20.04.06	<i>P.argentipes</i>	22	22	11.0
		<i>P.papatasi</i>	00		
		<i>Sergentomyia spp</i>	00		
Dharbhanga, PHC- Hayaghat	20.04.06	<i>P.argentipes</i>	08	18	9.0
		<i>P.papatasi</i>	10		
		<i>Sergentomyia spp</i>	00		
Madhubani, PHC- Sakari	21.04.06	<i>P.argentipes</i>	38	56	9.3
		<i>P.papatasi</i>	11		
		<i>Sergentomyia spp</i>	07		
Saran, PHC- Garkha	22.04.06	<i>P.argentipes</i>	79	79	11.0
		<i>P.papatasi</i>	00		
		<i>Sergentomyia spp</i>	00		
Vaishali, PHC- Patepur	10 & 11.05.06	<i>P.argentipes</i>	52	69	11.5
		<i>P.papatasi</i>	08		
		<i>Sergentomyia spp</i>	09		
Patna, PHC- Bakhatiapur	31.05.06	<i>P.argentipes</i>	50	50	12.5
		<i>P.papatasi</i>	00		
		<i>Sergentomyia spp</i>	00		
Arah, PHC- Arah	24.04.06	<i>P.argentipes</i>	26	43	7.1
		<i>P.papatasi</i>	10		
		<i>Sergentomyia spp</i>	07		
Araria, PHC- Araria	26.06.06	<i>P.argentipes</i>	20	20	10.0
		<i>P.papatasi</i>	00		
		<i>Sergentomyia spp</i>	00		
Kishanganj, PHC- Bhadurpur	27.06.06	<i>P.argentipes</i>	38	38	9.5
		<i>P.papatasi</i>	00		
		<i>Sergentomyia spp</i>	00		
Supaul PHC – Pipra, Supaul	01 to 03.07.06	<i>P.argentipes</i>	19	19	6.2
		<i>P.papatasi</i>	00		
		<i>Sergentomyia spp</i>	00		

Table 2: Post spray vector density observation



District	Date	Present status of sandfly		Total sandflies collected	MHD
Vaishali PHC – Patepur	02.06.06	<i>P. argentipes</i>	00	00	00
		<i>P. papatasi</i>	00		
		<i>Sergentomyia spp</i>	00		
Araria PHC – Araria	26.06.06	<i>P. argentipes</i>	17	17	8.5
		<i>P. papatasi</i>	00		
		<i>Sergentomyia spp</i>	00		
Kishanganj PHC Bhadurpur	27.06.06	<i>P. argentipes</i>	28	28	7.0
		<i>P. papatasi</i>	00		
		<i>Sergentomyia spp</i>	00		
Supaul PHC – Pipra, Supaul	01 to 03.07.06	<i>P. argentipes</i>	01		0.5
		<i>P. papatasi</i>	00		
		<i>Sergentomyia spp</i>	00		

21. Monitoring and supervision of DDT 50% spray operation in Kala-azar endemic districts.

Objective:

- Eradication programme of kala-azar initiated by Bihar Govt.

Progress:

Study of pre spray density

As per the assignment by NVBDCP, Govt. of India, this institute initiated monitoring of DDT spray and pre and post entomological surveillance in various districts endemic for Kala-azar viz. Muzaffarpur, Vaishali, Sithamarhi, Nalanda, Samastipur, Dharbhanga, Madubani, Saran, Patna, and Arah. Later on three more districts namely Araria, Kishanganj and Supaul were included. In Vaishali district (village, Belladam & Bella) spraying was conducted under our supervision.

After consultation and discussion with respective CS/DMOs, the team visited the villages where spraying operation was going on for monitoring. Sandfly density was measured. During the visit it was found that the spraying was not satisfactory. In many houses, only irregular patches of DDT were found on the sprayed wall. In some areas, no staff from the respective PHC was found available at the spot for supervision of the operation. The villagers were cooperative and some of them reported about unsatisfactory spray in their households.

- Total No. of villages visited : 113



- Total No. of households searched : 2414
- Total No. of Sandflies collected : 275
 - Total No. of *P. argentipes* : 253
 - Total No. of *Sergentomyia* : 22

DDT spray monitoring in an endemic village of Vaishali district



22. Validation of sandfly distribution and Kala-azar disease prevalence through Remote Sensing & GIS in endemic and non endemic foci of Kala-azar to reaffirm the earlier out come and its applicability for the entire Kala-azar endemic region of Bihar. (Sponsor: ICMR)

Objective:

- To correlate geographical distribution of sandfly (*P. argentipes*) in relation to visceral leishmaniasis and the satellite data obtained in respect of macro and micro system and other ground truth in the endemic and non-endemic areas evaluate its applicability in entire Kala-azar endemic area and its role as “**epidemic predictor**”.



Progress:

Under the ICMR task force project “Application of Remote Sensing in identifying and mapping sandfly distribution in endemic and non –endemic Kala-azar foci in Bihar”, it was observed that temperature, humidity, settlement/orchards, land areas of crop plantations, dry fallow, moist fallow and minimum normalized-difference vegetation index and standard deviation of NDVI are the main environmental variables independently associated with the vector density or presence of vector in endemic sites.

To further validate the outcomes at macro level, ground survey is being carried out at two levels i.e. district boundary level and village level. Boundary lines of each and every selected districts are being surveyed and sketched indicating the various important features viz. river, ponds, vegetation, forests, hills and information on various socio-economic parameters like total population, sex ratio, literacy, areas of water bodies, crop, settlement, peridomestic vegetations, trees, plants, cattle sheds are been collected and updated at village level.

Site preparation for Remote sensing



23. Early identification of asymptomatic cases of Kala-azar in endemic foci of Bihar, India: An epidemiological and socio-behavioral study.



(Sponsor: WHO/TDR)

Objective:

- To determine the asymptomatic cases and their conversion into disease stage.
- To study the socio-behavioral aspect of VL in the community

Progress:

This study was conducted in two villages namely Banthu and Haribanshpur of Vaishali district of Bihar on the basis of high endemicity of the VL cases as per the State Govt. data. Out of 1525 population (in 282 households) enumerated, 1017 were subjected for clinical examination, rk-39 dipstick test and PCR. The current and past history of case, clinical findings, diagnosis and treatment details were recorded.

Out of 1017 screened individuals, 97 were found positive by rK-39 and 31 were PCR positive in 345 blood samples tested so far. A total of 36 individuals were found for past history of kala-azar. All rk39 strip test / PCR positive cases are being followed on first week of every month. After 3-months follow up, out of 61 rk-39 positive (excluding past history of kala-azar), 21 developed as full blown kala-azar case and remaining 40 are still asymptomatic. The follow up has to be extended for one year to assess the proportion of asymptomatic cases and their conversion rate into disease stage.

A separate pre-tested semi structured questionnaire was canvassed to all the head of the households to elicit the information on socio-behavioral aspect in relation to disease, transmission, diagnosis, prevention and treatment seeking behavior. Data analysis on socio-behavioural aspect is under progress.

24. Magnitude of under-reporting of Visceral leishmaniasis (VL) cases in Bihar, India.

Objective:

- To determine the proportion of under-reporting of VL cases
- To estimate the actual annual incidence of VL cases

Progress:

Three highly endemic districts for kala-azar namely; Vaishali, Muzaffarpur and Samastipur have been selected based on State Govt. of Bihar data. Three most highly endemic PHCs



from each district have been selected. The first round information on details of the VL patients viz. name, age, sex, village, treatment etc. from each PHC is in process. Simultaneously, 10 villages from Lalganj PHC of Vaishali districts have been identified wherefrom VL cases are reported at the PHC. Door-to-door survey in one village namely, Banthu, has been initiated. The work is at preliminary stage that requires extensive door-to-door survey in the study area for cross-matching the cases recorded at the PHCs and actually found during survey; as well as assessment of the proportion of the under-reporting of the VL cases.

25. Implementation Strategies for visceral Leishmaniasis treatment in India (Sponsor: WHO/TDR)

General Objective:

To develop improved implementation strategies for early case detection and treatment of all VL patients.

Specific objectives:

- To determine the knowledge of, and the importance given to, leishmaniasis and its control by different sections of society
- To describe what public and private health services are available for VL care and to analyze their current practices for VL diagnosis & treatment
- To study people's health seeking behavior with respect to VL treatment and its determinants
- To determine the proportion of patients served by different health care providers and the proportion of patients never reported

Progress:

The survey covered a population 28343 in 5443 households spread over in 13 villages of an endemic Block in District Vaishali. On a sub sample basis, the total of 445 household interviews were conducted to elicit the information on Knowledge, Aptitude & Practice (KAP). During the survey, 61 fever cases (>2weeks) were subjected to rk-39 test and 31 were found positive. Patient interviews were conducted, and were referred to PHC/RMRI. Apart from this, a total of 69 Health Care Providers were interviewed in the study area. (Table 1)



Table-1: Important indicators

Total study population	28343
Total No. of Villages	13
Total No of households screened	5443
Total No of households interviewed	445
Total No. of current and past KA cases	56
Total No of fever cases of more than 15 days	61
Total No of fever cases positive for rk39	31
Proportion of rK39 positive cases positive for LD bodies	51%
One yr period prevalence of KA	2.0/1000 pop
Health Care Providers interviewed	69

Screening Schedule

A total of 5443 households were covered for screening wherein head of households were interviewed through pre-tested questionnaires. Age-sex distribution of the population screened shows that average age was 24% and mean SD was 18.9%. Proportion below 15 years of age was 40.4%, while 43% in the age group 15-45 years. The proportion of male was 53% and 43% in female. It was found that 40% of the individuals having past history of Kala-azar preferred Qualified Pvt. Doctors followed by Govt. Doctors (35%), Chemist (10%). Total number of fever history more than 15 days were 61 and all were subjected to rk39 test, out of which 31 were found positive.

Household Schedule

Head of the household (N = 445) for every 13th household during the survey and houses having past KA case or current KA case were interviewed for detailed Household schedule. The analysis shows that the mean age of the head of the household was 42.1% with SD 18.9. Among the total respondents 81% were male and 19% were female. Majority of the respondents were illiterate (44.8%) followed by middle or high school (26.87%), primary level (20.8%), and intermediate & above (7.6%). More than half were engaged in agricultural activities 33% as agricultural labour while 19% reported own agriculture, 15% were engaged in their own petty business and 11% were skilled labour. Mean number of living rooms were found to be 2.24 in the study area. As far as the house material is concerned 73.6% were hatched / Tati, 12.1% were mud plastered and 14.3 were brick un-plastered.

Kala-azar awareness



A very high Proportion 98.4% were aware of Kala-azar, but regarding symptoms the most common were fever 93%, chill/rigor 72%, blacking of Skin 9% and enlargement of abdomen 4%. Curable nature of disease was reported by 98.9%. Regarding transmission of the disease 80.2% reported by mosquito to bite, 10.4% reported through exterminated water and 13% had no knowledge. Knowledge regarding protection seems to be encouraging as 74% reported use of bed nets, 39% use of smoke, 26.9% repellents and 12% use of insecticide, around three fourth (73.6%) household reported having bed nets.

General health seeking behavior

For general health seeking behavior local unqualified doctor was the first preference with 82.9% of responses followed by private qualified doctor 56.9%, local chemist 41.2% and Govt. doctor 31.0%. Time required to reach the provider was 52 minutes for private qualified doctor and Govt. doctor and for the rest it is less than 15 minutes. The cost for reaching the private qualified doctor/Govt. doctor is around 50-55 rupees. As far as priority Health Care Provider is concerned the 1st priority for provider was local unqualified doctor with 49.7% respondents followed by private qualified doctor 39% and Govt. doctor 10% whereas in the second priority it is the private unqualified doctors (60.6%) and Govt. doctor (36.6%).

The main reason for selection of Health Care Provider in the first priority was proximity to the house (41.8%) and faith (37.9%) whereas in the second priority it is faith (60%) and cheaper (28.2%). Specifically in case of Kala-azar, the choice is private qualified doctor (44.7%) followed by Govt. doctor (33%) and unqualified doctor (21%). Here again faith (61.5%), cheaper (37.4%) and proximity to the house (18.7%) were the main consideration for selection of Health Care Provider.

Table-2: Availability of various health care providers with average time taken and expenditure in transportation

Characteristics	Percentage	Average	Average
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		time taken to travel (in mts)	money spent in reaching (in Rs)
Indigenous healer	3.9	11.4	0.10
Local chemist	41.2	14.5	0.17
Local unqualified doctor	82.9	13.7	1.30
Private qualified doctor	56.9	52.2	51.2
Govt. doctor	31.0	50.9	55.2

KA patient interviews

Duration between falling ill and seeking health care was reported by 2 weeks by 50% and more than 4 weeks 50%. Duration between going to health care provider and receiving the diagnosis was reported 3 weeks by 50% and more than 4 weeks by the rest of 50%. Duration between receiving the diagnosis and starting of treatment was reported <1 week by 25%, 3 weeks 25%, and more than 4 weeks by 50%. Paramedical were reported as the first contact by 50% of the cases while 25% contacted private medical doctor but for the second contact all have gone to private doctor. It was found that 75% cases were diagnosed by Aldehyde test and rest by bone marrow/splenic aspiration.

Table-3: Treatment seeking behavior and practices

Variables	Percentage
i. Interval between illness and seeking care	
< =1 week	0.0
2 weeks	50.0
3 weeks	0.0
=> 4weeks	50.0
ii. Interval between seeking care and diagnosis	
< =1 week	0.0
2 weeks	0.0
3 weeks	50.0
=> 4weeks	50.0
iii. Interval between diagnosis and starting of treatment	
< =1 week	25.0
2 weeks	0.0
3 weeks	25.0
=> 4weeks	50.0
iv. Diagnostic test as basis of treatment	
Bone marrow/splenic aspiration	25.0
Aldehyde test	75.0

26. In-depth review of current Kala-azar Programme.

(Sponsored: NVBDCP, Govt. of India and World Bank)

**General Objective:**

To review the implementation status of current Kala-azar control Programme.

Specific objectives:

- To review the disease surveillance.
- To review the access of early diagnosis and treatment.
- To review vector control strategies.
- To review IEC activities.

Observations:**Households Survey**

Quantitative data analysis reveal that a total of 8 districts and 12885 households covering 76942 population comprising 40565 male and 36377 female were covered for IDR Survey. Good proportion (45.87%) of the respondents was found illiterate and 55.09% houses were found Kuchha/Thatched. Among the surveyed household, 48.14% of houses got the cattle-shed inside or nearby the houses and 84.14% houses were having no toilet facility inside their houses.

Maximum surveyed population (27255) was from the age group 0-14 Yrs and 1380 (76942) were found Kala-azar cases, out of which 832 (60.29%) were male and 548 (39.71%) were female. Among the cases 121 (0.16%) were current cases and rest 1259 (1.6%) were past cases of Kala-azar. Thus the Prevalence and Incidence was observed as 17.93 and 1.57 respectively per thousand in the study area. Pregnancy was observed in 47 (0.20%) cases out of reproductive female age population (23172). There were 115 (9.13%) deaths reported out of 1259 past Kala-azar cases. As regard to diagnostic practices adopted for Kala-azar still diagnosis was mainly based on Aldehyde test /rk-39 test. Good proportion 622 (45.07%) of the Kala-azar cases prefer their treatment at Private Set-up. (Qualified Private Doctors, MBBS) As regard to IRS status only 5247(40.72%) households were sprayed with DDT. Two rounds IRS were reported by 1237 (23.58%) and out of 2nd round spray only 289(23.36%) households sprayed as per the schedule i.e. in the month of July- August.

Health Facility Survey

It was found that a total of 229 Health Facility Providers including medical officers, qualified private doctors, un-qualified private Doctors, District Malaria Officers, Malaria



Inspectors/Supervisors, health workers/ANM/MPW/BHV/LHV/BEE etc were interviewed. Among all the categories at Govt. set-up only 16.59% had attended some kind of KA training in last one year and all most all need further training/ orientation of Kala-azar. Generally the diagnostic practices adopted for the treatment of Kala-azar were rK-39 (58.89%) followed by Aldehyde test (33.33%) at Govt. Set-up as well as Private Set-up. Presently majority (23.33%) of the health facility providers at Govt. set-up start the treatment of Kala-azar on the basis of Aldehyde test followed by 15.55% on rK-39. It has been concluded from the findings that presently no diagnostic facilities were available at Govt. set-up except Aldehyde test. SAG was available at majority (77.55%) of the centers under Govt. Set-up followed by Amphotericin B (22.44%) for the treatment of Kala-azar. Among the Govt. health functionaries 26.53% fails to maintained buffer stock throughout the year.

Table 1: Distribution of Current and Past Kala-azar cases

State	District	HH Surveyed	Pop. Surveyed	Current KA Cases	%	Past KA Cases	%	Kala-azar cases	%
Bihar	East Champaran	1597	11405	23	0.20	197	1.73	220	1.93
	Muzaffarpur	1623	10961	18	0.16	135	1.23	153	1.39
	Saran	1672	12243	5	0.04	93	0.76	98	0.80
	Vaishali	1672	10105	11	0.11	150	1.48	161	1.59
Sub Total		6564	44714	57	0.13	575	1.29	632	1.41
U.P.	Ballia	1601	9725	7	0.07	103	1.06	110	1.13
Jharkhand	Godda	1378	6797	48	0.71	284	4.18	332	4.88
W.B.	24 Pargana (S)	1679	7551	1	0.01	243	3.22	244	3.23
	Murshidabad	1663	8155	8	0.10	54	0.66	62	0.76
Sub Total		3342	15706	9	0.06	297	1.89	306	1.95
Total		12885	76942	121	0.16	1259	1.64	1380	1.79

Table 2: IRS during current year and as per the scheduled month



State	Name of the district	No. of Household	No. of Household Sprayed	1st Round	2nd Round	IRS during scheduled month	
						Mar - Apr	July - Aug
Bihar	East Champaran	1597	303	281	22	0	1
	Muzaffarpur	1623	817	802	15	0	0
	Saran	1672	601	593	8	0	0
	Vaishali	1672	1213	756	457	20	62
Sub Total		6564	2934	2432	502	20	63
U.P.	Ballia	1601	25	23	2	0	0
Jharkhand	Godda	1378	1194	613	581	67	162
W.B.	24 Pargana (S)	1679	1003	851	152	28	64
	Murshidabad	1663	91	91	0	0	0
Sub Total		3342	1094	942	152	28	64
Total		12885	5247	4010	1237	125	289