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**Rajendra Memorial Research Institute of Medical
Sciences**

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INSTITUTIONAL STUDIES

1. Hospital based surveillance.

Institutional project

Objectives:

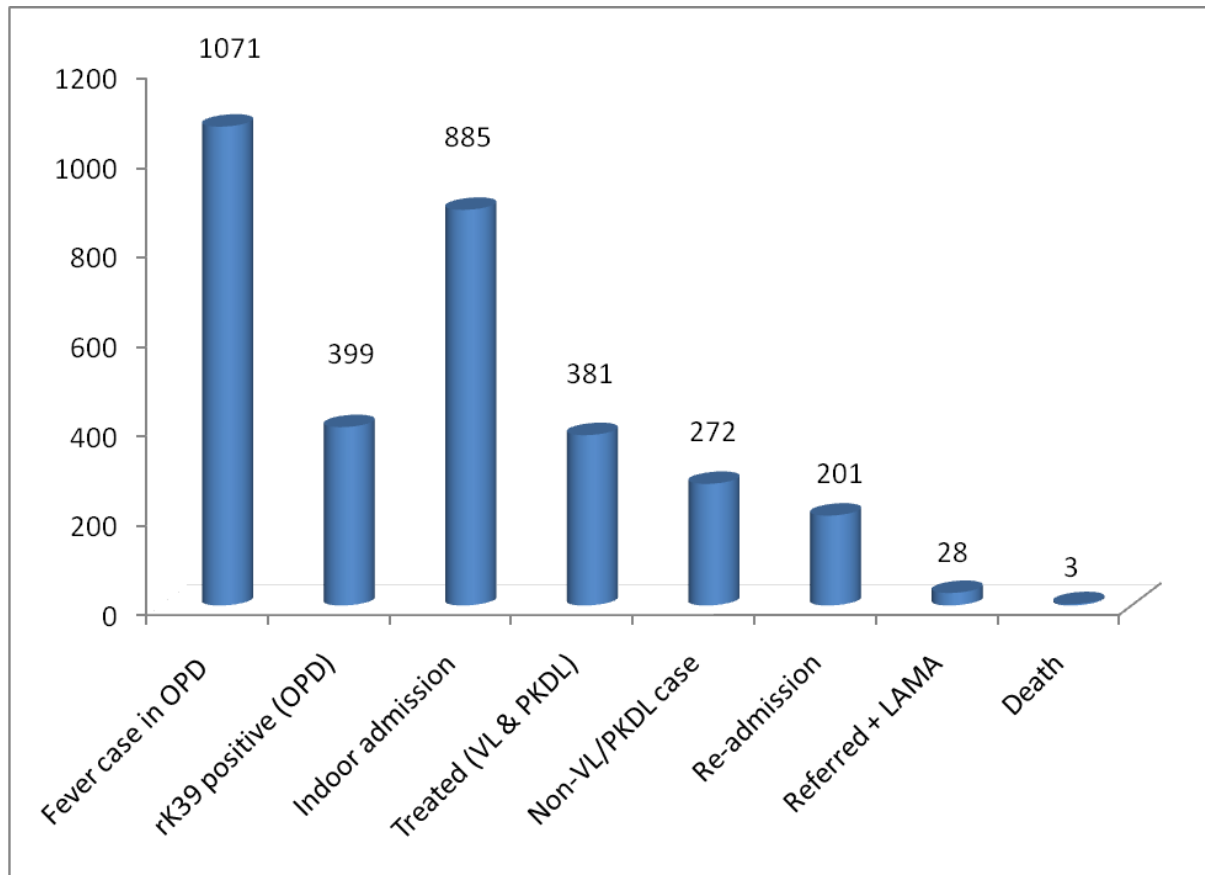
- To capture patient data at all the doorsteps.
- To interlink the patients' data, generated at various levels, in a comprehensive database
- To assist the Institutional scientists by providing patients related information instantly as per their research requirement.

Progress:

During the period Nov 2009 – Oct. 2010, a total of 8,658 new patients were registered in the out door patient department (OPD) for medical advise and treatment. A total of 1071 (12.4%) clinically suspected patients for kala-azar were first serologically tested with rK39 strip test in OPD, out of which 399 (37.25%) were found positive and advised for indoor admission for parasitological confirmation and treatment. Altogether patients' admission in ward reached to 885 during the period that also includes re-admission for follow up, relapse or non-kala-azar or PKDL cases. Comparatively high number of new admission records (399 vs 885; about 2-fold) may be attributed to direct referral by MSF, outside-diagnosed/ treated, relapse, follow up and PKDL cases who were not subjected for rk39 test.

Out of 885 total admission in Indoor, 381 (43%) were new cases of kala-azar and PKDL. They were either enrolled in the undergoing different clinical drug trials or treated as per the standard regimen of amphotericin B; rest were non-Kala-azar/ PKDL cases (272, 31%), re-admission for relapse, follow up or repeat course in case of PKDL (201, 22.7 %), referred or left against medical advice (28, 3.3%). A total of 3 patients died in the indoor. On an average, new registration in OPD, total admission in ward and new confirmed cases put on treatment came to 722, 73 and 32 respectively per month.

Table: A glance of suspected cases identified at OPD and Indoor status of the patients during the period Nov 2009 – Oct 2010 (Total New Regd. in OPD 8658)



2. Establishment of repository *Leishmania* parasites and sera bank at RMRIMS, Patna.

A.K. Gupta, P. Das et al

Aim and objectives:

The study aims to establish a repository / national resource of *Leishmania* parasites and sera sample which can cater parasites and sera sample of different groups to scientists/researchers working on various aspects of this disease. The objectives of this study are:

- To isolate *Leishmania* parasites from VL / PKDL patients having different characteristics such as clinical pictures, drug responses, geographical locations, etc., and from vector; their culture adaptation and cryopreservation in liquid nitrogen as well as *in vivo* maintenance of few selected important isolates.

- To cryopreserve different reference isolates of *Leishmania*.
- To characterize the various isolates.
- To preserve sera sample of KA/PKDL patients and suffering from different diseases, as well as of normal cases from different areas at -20°C.
- Proper documentation and archiving of all details.

Progress:

A total 89 different isolates of *Leishmania donovani* are being maintained in liquid Nitrogen after documentation of each isolates. One cryovial of each 5 different isolates of *L. donovani* (that were cryopreserved earlier), were revived. Four isolates are being maintained *in vitro* by sub-passaging in bi-phasic medium at the interval of 2-3 weeks. Six isolates were provided to scientists for their research projects.

In sera bank, 451 sera samples from various categories such as confirmed VL cases, relapsed VL cases, VL with HIV co-infection, PKDL cases, healthy endemic, healthy non-endemic etc have been preserved at -20⁰ C and are being utilized for different research works. Six different types of rapid diagnostic test kits have been evaluated for visceral Leishmaniasis using the sera samples of the sera bank under the project Visceral Leishmaniasis Laboratory Network (Supported by UNICEF/UNDP/World Bank/WHO/TDR.

Table: Details of sera samples stored in Sera bank

Category code	Category	# Sera samples
01	VL cases	51
02a	Healthy endemic with follow-up	39
02b	Healthy endemic without follow-up	80
06	Healthy non-endemic	152
07	PKDL cases	27
03	Relapsed VL cases	13
05	HIV-VL co-infection	05
04	Other disease (TB)	58
08	Others	26
	Total	451

INTRAMURAL STUDIES

Epidemiology

3. The economic burden of Visceral Leishmaniasis at the household level in Bihar, India.

A Ranjan et al.

Aims and Objectives:

Qualitative Research

- To identify the factors affecting the income and expenditure pattern at household level in Kala-azar affected households in rural areas through FGDs and in-depth interview of VL affected HH.

Quantitative Research

- To estimate the economic impact at the household level by measuring direct and indirect cost for VL care
- To investigate coping strategies to pay for VL care

Progress:

Paroo PHC was identified as the highest endemic PHC of VL in Muzaffarpur district during 2008 based on the records obtained from the concerned District Malaria Officer. After going through the PHC records of VL cases in the year 2008, it was found that two villages namely, Bangra and Deoria, were most affected and eventually the affected households of these two villages were identified. A sampling frame of potential participants for conducting two focus group discussions (FGD) were created, and 12 participants were selected after narrating the purpose of the study. Date, time and venues were fixed prior to FGDs. Two FGDs were conducted by PI as moderator and two investigators as coordinators. Proceedings were tape-recorded and notes were taken by investigators. Later on, proceedings were transcribed using tape-recorded and notes.

For conducting in-depth interviews, a sampling frame of potential participants was created from the records of these two villages. Apart from this, households having past history of VL in previous year were also identified by the team. Twenty participants were

selected from the two villages after getting their consent. Three investigators conducted in-depth interviews using a common guideline.

Based on the results of qualitative study, structured questionnaire was developed and modified to collect data from 250 VL cases in high endemic areas of kala-azar. The study is in progress.

4. Spatial and seasonal distribution of Kala-azar cases in Bihar, India.

NA Siddiqui et al.

Objectives:

- To evaluate the average amplitude of kala-azar cases across the seasons and to observe the seasonal trends.

Specific objectives

- To measure the average annual incidence of Kala-azar.
- To measure the magnitude of seasonal variation of kala-azar incidence.
- To measure the magnitude of geographical distribution of Kala-azar incidence.
- To measure magnitude of patients-visiting rates to public sector facilities in different seasons.

Progress:

The district and year wise retrospective data of reported cases and deaths of Kala-azar of all the 39 districts of Bihar for the period of 2001 – 2007, available at the State Health Department, Bihar and National Vector Borne Diseases Control Programme, India were collected, compiled in a database and analyzed. It was observed that in seven years period a total of 1,41,650 kala-azar cases were treated in the Public Sector with 1116 deaths. The annual incidence of kala-azar varied from 1.14 – 4.56 per 10,000 population (Mean 2.4) and the cause specific deaths rate varied from 1.3 to 2.5 per 10,00,000 (Mean 1.8). There was an increasing trend of annual incidence but as compared to 2001, significant increase (about 2.5 – 3 folds) was observed from 2005. The cause specific death was slightly on higher side since 2001 to 2003 but 2004 onwards it was almost consistent.

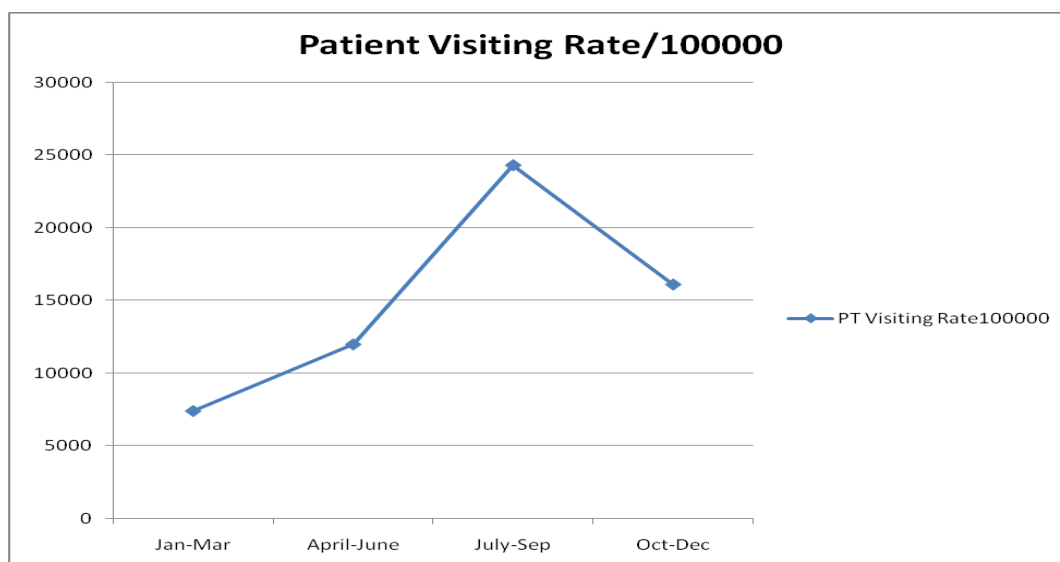
Out of total 141650 cases, 88485 were registered between two quarters i.e. April – June and July – Sept. contributing about 63% of total cases. The highest average annual

incidence (0.74/10000) was observed in July – Sept. quarter while it was lowest (0.40/10000) in Oct – Dec. The average annual incidence of April – June and July – Sept quarter was found consistent (0.70/10000).

The geographical distribution of kala-azar cases revealed significantly higher proportion of cases (75589, 53%) in North areas of Bihar followed by East (58283, 41.5%), South (4160, 3%) and West (3618, 2.5%). The average annual incidence was significantly higher (3.9/10000 and 2.8/10000) in the East and North areas respectively as compared to West and South (0.33/10000). The average cause specific death was highest (2.7/10,00,000) in the North areas whereas it was more (1.3/10,00,000) in West areas as compared to East (1.1/10,00,000) in spite of the lower average annual incidence of the disease.

To explore general health seeking behavior bias, we examined trends in the total patients visits in general (OPD attendance) to public health system in all the districts of Bihar and it was found 5,70,48,742 over seven years period. The annual OPD attendance varied from 59,71,091 to 1,11,40,984 (Mean 81,49,820). There was an increasing trend of OPD attendance over all.

Seasonal distribution of OPD attendance to public sector facilities revealed that July-September quarter contributed maximum OPD attendance (2,31,85,141) followed by April-June (1,14,35,266). Patients visiting rates were more than double between July to September quarter than between April-June (24,293 vs. 11,982 patient visits per 100 000 population). Similarly, the patient visiting rate was highest in North areas (24,124) and lowest in West areas (11,352). The patients visiting rates were about double in North areas than East areas (24,124 vs. 12,740 patient visits per 100 000 population).



5. Parameters associated with progression of asymptomatic to symptomatic VL cases.

R.K. Topno et al.

Objectives:

- To identify early indicators e.g. hematological picture and its relation in progression of asymptomatic to symptomatic kala-azar
- To identify the relation of the immunological marker like IL-10, Interferon- γ in progression of the Kala-azar cases from asymptomatic status.

Progress:

Based on Kala-azar incidence during last two years as per the Govt. data, two villages viz. Nandanketuka and Narayanpur of Parsa PHC under Saran district were selected. A door-to-door survey was conducted in both the villages wherein the head of the households were interviewed using pretested structured questionnaire to collect data on epidemiological profile of the incumbents. The subjects who did not had any sign and symptoms and past history of kala-azar were serologically screened with rk39 strip test to identify the asymptomatic cohort having leishmania infection. About 2 ml venous blood samples were collected from the asymptomatic subjects for immunological study. All the asymptomatic subjects were clinically examined at every two months follow up visits to observe conversion into full-blown case of kala-azar. Till date, about 6% individuals were identified as asymptomatic, out of which only 3% converted to full blown symptomatic cases.

Total Population Screened	1253
rK39 Test Done	1017 (81.2%)
Male	456 (44.8%)
Female	561 (55.2%)
rK 39 Test Positive (Asymptomatic cases)	63 (6.2%)
Conversion of asymptomatic to symptomatic	2 (3.2%)

6. Quality of life of Visceral leishmaniasis (VL) patients in Bihar; India.

A. Ranjan et al.

Objectives:

- To assess the quality of life among the VL patients comparing with healthy controls matched for age and sex in neighbourhood
- To compare the quality of life of VL patients before and after therapy
- To assess the duration to attain level of QOL equivalent to healthy controls

Progress:

As per the SAC recommendation, the sample size of the study has been recalculated. Earlier it was 65 subjects, after recalculation, it came about 90 subjects for assessing change in QOL of VL patients before and after therapy. Generic and patient questionnaires were developed to collect information on sub-domains of quality of life such as physical functioning, role functioning, social functioning, cognitive functioning, emotional functioning and global quality of life using standard scales for both case and control subjects. Both the questionnaire were pre-tested after collecting data of 20 VL patients admitted in the clinical ward of RMRI.

It was observed that there were lots of non-responses for many items in the questionnaire. Some of the items were not well understood by the subjects in course of interview. There is a need to re-designe a proper questionnaire for the collection of data. After thorough web search, a set of questionnaire, “WHOQOL-HIV Instrument” was found to be the best as far as simplicity and scope of response is concerned. This questionnaire will be modified as per the requirement for collection of data. The study is in progress.

7. Association of HLA Class I and Class II Alleles in susceptibility to Visceral leishmaniasis in endemic and non endemic regions of Bihar.

D. Singh et al

Objectives:

- To investigate the genetic diversity of HLA in person having kala-azar from endemic and non endemic regions.

- To determine the HLA class I and class II allelic distribution in the patients and healthy controls.

Progress:

About 200µl peripheral blood samples were taken from clinically diagnosed and rk39 positive kala-azar patients coming from different endemic & non-endemic region of Bihar. Blood samples were also collected from healthy contact persons those who had no previous history of kala-azar and without clinical signs and symptoms as well as serological negative for rK39 strip test. Five unrelated healthy control samples from endemic and non endemic region were also included, which had no previous history of kala-azar in their houses, without clinical signs and symptoms and serologically negative by rK39 strip test.

Collected blood samples were subjected to DNA isolation using Qiagen blood mini kit. PCR was performed by using primers of HLA-A, HLA-B, HLA-C (Class-I) and HLA-DRB1, HLA-DQB1, HLA-DPB1 (Class-II) locus. PCR products were analyzed by electrophoresis on agarose gel and were found 2 Kbp (HLA-A, locus); 1.2 Kbp & 1.5 Kbp (HLA-B, locus); 1.2 Kbp (HLA-C, locus); and 365 bp (HLA-DRB1, locus), 400 bp (HLA-DQB1, locus), 400 bp (HLA-DPB1, locus), band respectively. Amplicons were purified by using ExoSAP-IT and subsequently cycle sequencing reaction was performed using internal primers provided with Abbott, HLA kit. Sequencing was performed in ABI 3130xL Genetic analyzer and results were analyzed by HLA analysis software (Conexio Analyzer).

Our result showed that of HLA-A *02010101 and HLA-DRB1 *150201 alleles associated with protection against visceral leishmaniasis, while HLA-A *020601 & *24020101 and HLA-DRB1 *150101 alleles are associated with susceptibility to visceral leishmaniasis. Work is under progress.

8. An epidemiological study to assess the prevalence of PKDL in endemic areas of Bihar.

V.N.R. Das et al.

Objectives:

- To determine prevalence of PKDL in an endemic community of Bihar.
- To assess management of PKDL cases in the community
- To establish intra-familial transmission

Progress:

The study was carried out in Rukhai village of Chandi PHC wherefrom regular occurrences of VL cases have been reported. Head (in absence any adult member) of the households were interviewed during door-to-door survey using structured questionnaire to collect information on socio-demographic characteristics like age, sex, occupation, and past history of VL and treatment. Each suspected PKDL cases were clinically and physically examined for detection of any skin lesions.

The total population of the Rukhai village was 223 having 116 male (52%) and 107 females (48%). Out of 223 individuals, 41 had past history of VL occurred during 2001 to 2007, with maximum number of cases in 2006. Out of 41 cases, 40 cases were treated with recommended dosage of Sodium Antimony Gluconate (SAG), and only one case received recommended dosage of Miltefosine. All of them got cured with no history of further relapse. A total of 11 individuals (male-5, female-6) were clinically and serologically identified as PKDL cases.

The survey was further expanded in the case-based selected villages of Forbesganj block, Dist. Araria districts. Out of 23,915 population from 4323 households, 12 clinically diagnosed PKDL cases (Male 5, Female 7) were detected. All 12 subjects were subjected to rK39 test. Out of 12, 9 had past history of VL (all were rK39 positive). All the identified PKDL cases were referred to the concerned PHC for treatment. The work is under progress.

Diagnostics**9. Study of Imprint smear microscopy and PCR application on biopsy from dermal lesions for diagnosis of Post kala azar dermal leishmaniasis cases in Bihar.**

N. Verma et al.

Objectives:

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

Progress:

After clinical examination for lesions' type, site and coalescence, biopsies from different skin lesions were collected aseptically from 80 PKDL cases (50 fresh, 20 after treatment and 10 follow up cases). Imprint smears were prepared for detection of leishmania parasites under microscope. Biopsy samples were collected in Tris buffer solution for PCR study. PCR detection of whole ITS region of the ribosomal RNA (rRNA) gene was done. DNA was isolated by commercially available 'QIAamp DNA tissue mini kit (Qiagen). A nested PCR has been developed from biopsy samples to amplify the ITS region of rRNA gene of *L. donovani* from previously amplified PCR product to increase the sensitivity and specificity. The amplified reactions were visualized on 1.5 % agarose gel using a DNA marker. The positive samples showed positive ~1100 bp band, whereas negative samples showed no band. Parasitological (L.D.) positive sample was used as a positive control. As a negative control, skin biopsies from 10 known patients of fungal diseases or leprosy were collected.

The comparative analysis of both these tools revealed that leishmania parasite positivity by PCR and microscopy were 95.6% and 91.3 % respectively in papulonodular lesions whereas in case of hypopigmented macular lesions, PCR was found to be more sensitive (92.5%) than microscopy (44.4%). The sensitivity of the PCR was found 24% higher than microscopy. Among the 20 treated PKDL cases, 2 were found positive by PCR after first course of treatment, but microscopically they were negative, possibly due to very high sensitivity of PCR to detect even very low amount of parasitic DNA. The study and analysis is in progress in after treatment and follow up cases of PKDL.

Clinical

10. Study of clinical and laboratory parameters as a predictive value for treatment failure with different anti-leishmanial drugs.

Nawin Kumar et al.

Objectives:

- To determine predictors of treatment failures by different anti-leishmanial drugs based on clinical and laboratory parameters.
- To compare initial cure and final cure by different parameters at 6 month follow up.

Progress:

Fresh and parasitologically confirmed VL cases from both sexes admitted in the indoor ward were enrolled in this study. The clinical parameters used in this study were demography and duration of illness before the start of anti-leishmanial drugs. Size of spleen was measured at day 0, weekly and at the end of therapy as well as during follow up at 1 and 6 month.

Laboratory parameters include Hb%, Total and differential W.B.C. count, platelet count, serum albumin, serum amylase, liver and renal function tests along with few additional parameters such as CRP, serum folate, ferritin, transferrin, iron, apolipoprotein A1 and ApoE and triglyceride were assessed at day 0, weekly and at the end of therapy and during follow up at 1 and 6 month .

It was observed that albumin, transferrin, iron, Apo A1, TIBC, Hb% and platelet counts were down regulated at the start of the therapy. Alpha-amylase was found increased in few VL patients which suggest about the involvement of pancreas during VL infection. The entire above mentioned laboratory parameters improved during the course of therapy with different anti- leishmanial drugs. It was observed that transferrin, albumin, iron which were down regulated can act as a predictive parameter in the assessment of drug response, while alpha amylase can be suggestive of complication related to pancreas and can lead to drug failure in latter course.

Till now we have enrolled 35 fresh VL cases in this project, of which 23 were treated with Ampho B and 12 cases with Miltefosine.

So far as assessment of predictive value of clinical and laboratory parameters on other anti-VL drug like SAG is concerned, this drug is not in regular practice for admitted VL patients of the Institute and the retrospective data does not incorporate all the parameters as per the study protocol.

Table 1: Laboratory parameters at different time points (N=35)

Parameters	0 Day	7 Day	14 Day	21 Day	EOT	1-month Follow up	6 month Follow up	Normal
Albumin	1.0 – 3.4	2.7 – 4.3	3.3 – 4.5	3.4 – 4.7	3.7 – 4.9	3.5 – 4.9	3.8 – 5.0	3.2 – 5.0
Amylase	59.0 – 179.0	58.0 – 189.0	80.0 – 186.0	70.0 – 191.0	63.0 – 169.0	70.0 – 156.0	68 - 120	<100
Transferrin	93.0 – 172.0	90.0 – 186.2	99.7 – 181.6	117.1 – 168.3	120.5 – 158.2	165.0 – 230.0	174.0 - 278	200.0 – 380.0
Iron	42.9 – 64.8	49.0– 68.2	44.9 – 69.0	48.9 – 69.0	51.2 – 74.2	60.5 – 102.0	62.8 – 138.0	65.0 – 175.0 (M) 50.0 – 170.0 (F)
Apo A1	71.0 – 132	88.0 – 135.1	92.0 – 138.2	98.8 – 138.9	101.2 – 138.8	110.1 – 140.8	114.2 – 150.6	122.0 – 161.0
Triglyceride	88.0 – 183.8	58.0 – 258.0	40.0 – 168.0	55.0 – 177.0	54.0 – 148.0	72.0 – 146.0	76.0 - 148.8	<150
TIBC	133.3 – 243.5	129.0 – 237.0	142.5 – 254.2	147.9 – 244.1	162.2 – 231.2	176.5 – 252.3	186.8 – 286.8	200.0 – 400.0
Transferrin saturation %	22.1 – 48.0	26.1 – 50.0	24.8 – 45.7	26.3 – 44.7	26.9 – 39.9	27.8 – 41.0		-
Hb%	6 – 10.7	7.8 – 10.8	8.9 – 10.8	9.0 – 11.8	10.0 – 11.8	11.8 – 13.6	11.9 – 14	10.0 – 14.0
TC	1650 – 9990	4260 – 8900	4800 – 1000	5200 – 10600	6300 – 10960	6150 – 11000	6450 - 11300	4000 – 11000
Platelet	76000 – 180000	78000 – 215000	120000 – 260000	200000 – 267000	215000 – 288000	216000 – 298000	238000 – 334000	150000 – 350000

11. Susceptibility to Visceral leishmaniasis in human beings – the role of testosterone.

K. Pandey et al.

Objectives:

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

Progress:

A total of 22 male subjects were screened for their level of testosterone, out of which 18 were confirmed VL cases and 4 were healthy controls. The VL cases were categorized into low, moderate and severe parasitic infection as per their parasitological assessment (following the WHO Leishmania amastigote burden) of 1+, 2+ to 3+ and >3+. The average testosterone level in VL patients was 2.410 ng/ml whereas in the control group it was 10 ng/ml suggesting a considerable decline of testosterone level subsequent to *L. donovani* infection. The relation of testosterone level with parasitological load was analyzed and it was observed that whenever the parasitic load increased there was a sequential decline in the level of testosterone. The effect of testosterone imbalance on progression of the disease was further evaluated through its association with IL-10 production during *L. donovani* infection.

Table 1: Evaluation of level of testosterone

Category	Number	Average Level of testosterone (ng/ml) (Inhibition ELISA)
VL cases	18	2.410
Healthy Control	4	10

Table 2: Effect of testosterone imbalance on progression of disease (IL-10)

Category	Number	IL-10 (pg/ml)
VL cases with high testosterone	7	20
VL cases with low testosterone	3	19.66

12. A study to assess the safety and efficacy of zinc supplementation in treatment of Visceral Leishmaniasis (VL) in Bihar.

VNR Das et al.

Objectives:

- **Primary Objective:**
 - To evaluate the efficacy and safety of zinc supplementation along with the anti-leishmanial drugs in achieving an initial cure

- **Secondary Objectives:**

- To evaluate the efficacy and safety of zinc supplementation along with the anti-leishmanial drugs in achieving a final cure rate
- To evaluate parasite clearance at different stages through RT-PCR.
- To evaluate the Th-1 (IL-12, IFN- γ , TNF- α) and Th-2 (IL-10) immune response during the course of treatment.

Progress:

The parasitologically confirmed Zinc deficient VL cases meeting all the inclusion and exclusion criteria were randomized in two arms. A total of 27 subjects were enrolled in the study, out of which 16 subjects in test arm and 11 in control arm. The patients under test arm were treated with amphotericin B in the dose of 1 mg/kg body weight for 15 infusions on alternate day along with zinc supplementation in the form of zinc Sulphate tablets in the dose of 200 mg twice a day whereas the patients under control arm were treated with amphotericin B alone in the above-mentioned dose.

Laboratory investigations were carried out on 0 day, 15 day and 30 days. In test arm all the patients were initially cured whereas in control arm one patient was found parasitologically positive at the end of treatment (Initial cure rate 91%). In both arms, blood urea and serum creatinine was found slightly raised in few cases (3 in Test arm and 4 in control arm), but it was not clinically significant.

Immunological profile of patients under test arm revealed that level of IFN- γ considerably increased. In some patients the increase was quite evident at Day 14. IL-10 levels were quite high at Day 1 which dropped steadily with treatment. In some cases, it maintained a stationary phase after Day 14. Interestingly, TNF- α level raised high at Day 14 but significantly dropped thereafter and almost reached to very low level at Day 28. ELISA and intracellular FACS of cytokines revealed that level of IFN- γ was increased from 35.07 pg/ml to 572.07 pg/ml and level of IL-10 decreased from 768.85 pg/ml to 111.92 pg/ml. Surprisingly, levels of TNF- α raised at Day 14 but dropped considerably at Day 28. PCR test at different interval suggests early cure in test arm but more number of cases need to be studied to reach at any conclusion.

13. Safety and efficacy of a combination of Amphotericin B and Miltefosine compared to Amphotericin B alone in patients with Post Kala-Azar Dermal Leishmaniasis (PKDL) - An Observational Pilot Study

K. Pandey et al

Objectives

- *Primary Objective:*
 - To evaluate the efficacy and safety of combination drug Miltefosine (28 days) and Amphotericin B (15 infusions) for the curative potential cure (parameter: rate of patients with nodular and papular lesions who achieve negative parasitology 12 months after end of treatment).
- *Secondary Objectives:*
 - To assess the rates of relapse after initial response
 - To evaluate the biochemical and hematological parameters during the course of treatment.

Progress

Under this study, 3 parasitologically confirmed PKDL cases with mixed type of skin lesions were enrolled in the test arm and 3 in the control arm under this study. All the three patients, under test arm, were administered Amphotericin B in the dose of 1 mg/kg body weight for 15 infusions on alternate day followed by miltefosine in the dose of 2.5 mg/kg body weight per day in divided doses for 28 days orally. The patients under control arm have completed 1st course of amphotericin B in the dose of 1 mg/kg body weight for 15 infusions on alternate day. Mild improvement in the skin lesions were observed both in the test as well as the control arm without any adverse events. The 3-months and 6 months follow up has not yet been completed. The study is in progress.

Basic

14. Crucial role of plant's extract in propagation of *Leishmania donovani* promastigotes.

A.K. Gupta et al.

Objectives:

To explore the possibilities of some plants' extract as a source for replacement of FCS/serum/ blood/ blood products in routine culture of *L. donovani* promastigotes.

Progress:

Assessment of Vacuum dried plants' extract for in-vitro propagation of promastigotes

The processing procedure to get plants' extract may affect the ingredients which have been found beneficial for propagation of *Leishmania* promastigotes in long term continuous successive sub passaging. To assess whether vacuum dried plants' extract retain its proliferation-promoting activity, medium supplemented with vacuum dried plants' extract were used in experiments. Same medium with FCS was taken as positive control and plain medium was taken as negative control. It was found that *Leishmania* promastigotes propagated well in medium supplemented with vacuum dried plants' extract and FCS in long term continuous successive sub passaging suggesting that it retains the capability to propagate *Leishmania* promastigotes in long term continuous successive sub passaging.

Thus, obligatory growth factors did not lose during processing and this completely animal products free culture medium could satisfy all those various functions and nutritional contributions that FBS/blood provides to support growth and reproduction of promastigotes.

Quantification of promastigote growth in media supplemented with plants' extract

In previous experiments, adequate number of promastigotes was observed when long term continuous successive sub passaging was done in medium supplemented with various plants' extract. But, it was not known whether parasite number in plants' extract supplemented medium is quite enough or at par to FCS supplemented medium or not. To clear this fact, medium was supplemented with randomly selected extract of 2 plants (that had shown propagative effect previously) in 3 different concentrations (i.e. 20%, 10% and 5%). Medium with FCS (10%) was taken as control. These media were inoculated with 1×10^5 cells / ml. Total number of cells was counted at every 24 hrs.

In medium supplemented with one plant extract at 20% and 10% concentration, maximum growth of promastigotes crossed 1×10^8 cells / ml) on 6-7th day, quite enough for mass culture in a short period and routine culture at extremely low cost. At 5% supplementation of plants' extract, the maximum growth crossed 5×10^7 cells / ml on 7th days. This concentration may be used for routine culture of promastigotes. Survival of promastigotes was observed at least 7 weeks in all.

In medium supplemented with another plant extract at 20%, maximum growth crossed 1×10^8 cells / ml and in 10% concentration, it was comparatively less (1×10^8 cells/ ml) on 6th day. Survival of promastigotes was observed till 8 weeks and 7 weeks at 20% and 10% concentration respectively. At 5% supplementation of plants' extract, maximum growth crossed 3×10^7 cells/ml on 8th day. Survival of promastigotes was observed at least 2 weeks. Hence, sub culture of promastigotes may hardly be recommended at this concentration.

Optimization of inoculums size for adequate propagation

Three different concentrations of promastigotes i.e. 1×10^4 , 1×10^3 and 1×10^2 cells/ ml were inoculated in medium having 10% of plant's extract. Counting of promastigotes was done on 3 successive days starting from 7th day, because maximum growth was observed on 6-7th day with higher inoculum (i.e. 1×10^5 cells / ml). Total number of promastigotes/ml on 9th day was > 3 times, 1.5 times when the inoculation size was 1×10^4 , 1×10^3 cells/ml and it was 0.5×10^7 cells/ml in case of inoculums size of 1×10^2 cells/ml. The promastigotes were very active and growth pattern was in increasing trend. Hence, medium with plant's extract is also quite enough sensitive to support propagation of promastigotes even at low inoculums size and may produce adequate number. Thus, the medium may be used in routine *in vitro* maintenance of different isolates.

15. Search for anti-leishmanial activity in crude plants' extract.

A. K. Gupta et al.

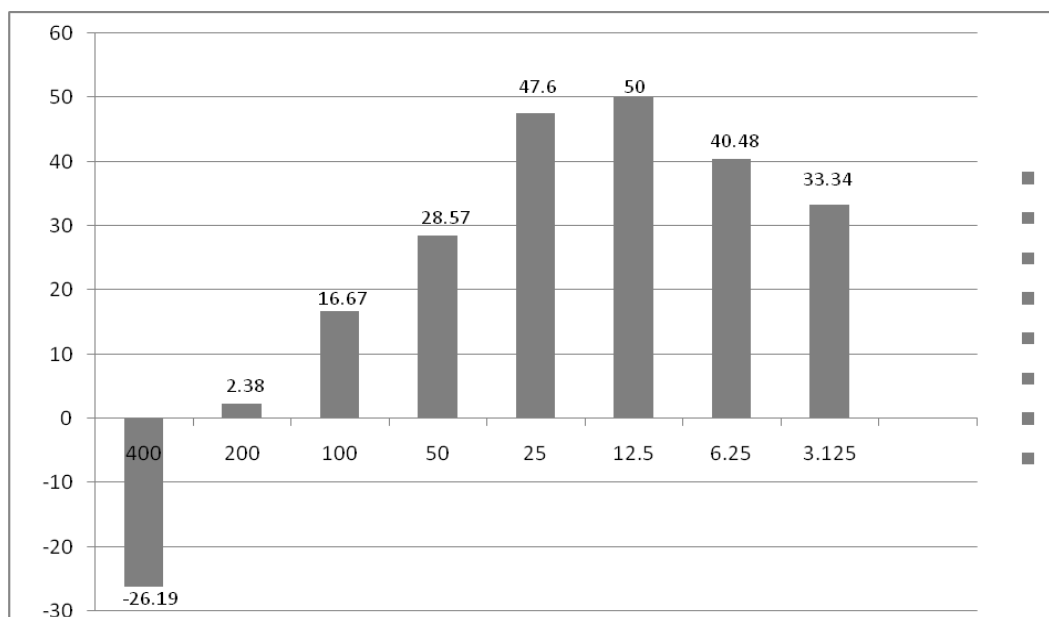
Objectives:

- To explore possibilities of crude plants' extract as a source for antileishmanial compound

Progress:

During screening of plants' extract for propagative effect of promastigotes, 3 of them caused 100% mortality of promastigotes in RPMI-1640, SIM and LGPY medium, even after addition of 10% FCS in these culture media after exposure of 72 hours. Hence, it was worthwhile to check for anti-leishmanial compound in these plants' leaves. One (non-traditional medicinal plant) of these plants which leaves are usually consumed as vegetable was taken for study.

Extraction was done from powdered leaves with ethanol (100%). To determine the antileishmanial effect on promastigotes, different concentrations of this fraction was evaluated.



Positive value indicates growth inhibition percentage & negative value correspond to growth stimulating percentage. Preliminary results showed that IC⁵⁰ of crude ethanolic extract was 12.5µg/ml. This observation may give a positive lead to search out anti-leishmanial compounds in the leaf of this plant.

16. Studies on some nutritional factors in severity of Visceral Leishmaniasis (VL).

C.S. Lal et al.

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:

In order to assess the nutritional markers in the malnourished VL patients, till date we have examined albumin, total protein, zinc, copper, iron, total iron binding capacity, transferrin, folate, calcium, magnesium, cholesterol, triglyceride, HDL, LDL and VLDL.

Decreased cholesterol and increased triglyceride has been observed in malnourished VL patients and is directly correlated according to BMI index. Albumin was down regulated as the BMI index decreases. The results of calcium did not showed any difference in both the groups (malnourished VL and Control VL). Total protein showed significant lower trend as the BMI index decreases. During the investigation, quality control sera were also analyzed. The external quality control measures were also taken into account during the experiment.

As regards the severity of the disease, the nutritional markers were studied in chronic and acute VL cases. The trend of down regulation of zinc was observed as the severity of the disease increases. Serum Zn level showed more decreasing trend in chronic cases as compared to acute ones. Serum Mg was significantly higher in chronic cases as compared to healthy ($p=0.006$) but almost similar in acute VL and healthy individuals ($p=0.928$). There was no significant difference between serum Cu, Fe and Ca. As regards the severity of the disease the following nutritional markers were assessed in chronic and acute VL cases. The results are shown in Table.

Table: Comparison of levels of trace elements of chronic and acute visceral leishmaniasis patients and control group

Variables	Chronic VL Patients (1) (n=22)	Acute VL patients (2) (n=22)	Healthy Controls (3) (n=22)	p-value for ANOVA	p-value for Tuckey's Test
Cu(µg/dl) Mean (95% CI)	384.94 (348.39 – 421.49)	332.80 (279.21 – 386.40)	111.41 (101.96 – 120.86)	0.001	0.114 (1 vs. 2) 0.001 (1 vs. 3) 0.001 (2 vs. 3)
Zn(µg/dl) Mean (95% CI)	44.51 (42 – 47)	56.27 (48.83 – 63.71)	78.04 (72.63 – 83.45)	0.001	0.007 (1 vs. 2) 0.001 (1 vs. 3) 0.001 (2 vs. 3)
Fe (µg/dl) Mean (95% CI)	42.86 (39.20 – 46.52)	40.61 (37.33 – 43.88)	93.62 (85.53 – 101.72)	0.001	0.817 (1 vs. 2) 0.001 (1 vs. 3) 0.001 (2 vs. 3)
Ca (mg/dl) Mean (95% CI)	9.64 (9.14 – 10.15)	9.11 (8.62 – 9.58)	9.79 (9.33 – 10.25)	0.096	0.238 (1 vs. 2) 0.889 (1 vs. 3) 0.098 (2 vs. 3)
Mg (mg/dl) Mean (95% CI)	2.30 (2.14 – 2.47)	1.96 (1.82 – 2.10)	1.99 (1.87 – 2.12)	0.001	0.002 (1 vs. 2) 0.006 (1 vs. 3) 0.928 (2 vs. 3)

17. Study on cholesterol involvement in Visceral Leishmaniasis.

C. S. Lal et al.

Objectives:

- To determine biochemical constituents (APRs and others) influencing cholesterol in VL patients.
- To examine about the effect of cholesterol in the growth and virulence of leishmanial parasites *in vitro*.
- To determine cytokines influencing LCAT synthesis.
- To assess lipoprotein metabolism influencing cholesterol

Progress:

Initially twenty (20) VL and healthy controls were taken in this study during the period. The parameters studied were total cholesterol, HDL-C, triglyceride, VLDL, LDL-C,

Apolipoprotein A1 (Apo A1) and B (Apo B) were assessed. The study revealed that total cholesterol, HDL-C, LDL-C and Apolipoprotein A1 was down regulated in all the VL patients but triglyceride was raised in VL patients while Apolipoprotein B was normal as to the healthy control before the start of the treatment. However, at the end of the treatment all the parameters returned to normal healthy control value except HDL-C and Apolipoprotein A1 (Table).

Parameters (mg/dl)	VL patient's result		Normal Healthy Control
	Before treatment	After treatment	
Total cholesterol	45.7-65.3	124.9-154.4	135.5-227.0
HDL-C	5.6-14.5	23.0-28.0	28.8-38.2
triglyceride	187.7- 274.4	94.0-150.1	98.5-155.0
VLDL	37.5-54.8	18.8-30.0	19.7-31.0
LDL-C	20.3-28.7	76.9-89.0	68.4-165.5
Apo A1	48.4-56.0	109.3-115.0	118.0-158.1
Apo B	59.2-78.5	78.0-88.4	74.8-117.5

18. Identification of Anemia as pathogenic factor in Visceral Leishmaniasis.

S. Narayan et al.

Objectives:

Primary objective

- To understand the role of *Leishmania* parasite in causing anemia in visceral leishmaniasis.

Secondary objectives

- To estimate the erythrocyte number, haemoglobin levels in primed and non-primed blood samples.
- To estimate Heme and Non-Heme Iron concentration in parasitic exposed and non-exposed blood samples.
- To estimate attached haemoglobin in the flagellar pocket of parasite.

Progress:

After standardization of technique for hematological estimation, the human (Kala-azar patient and healthy subjects) erythrocytes (1×10^8 /ml) were washed three times with phosphate buffer saline with 1% glucose (PBG) and incubated with equal volume of parasites at 25° C for a required period of time. The red cells without parasites were incubated in assay buffer. After completion of incubation the hematological analysis of RBC & Hb were done through cell counter in both the samples.

After standardizing the technique for estimation of heme & non-heme iron contents in non-primed and *L. donovani* primed VL blood samples and control, the erythrocytes culture was done. Incubated cells were lysed by adding lysing buffer and then formic acid was added. The estimation of heme content was done spectrophotometrically by reading the absorbance at 398 nm in the presence of standard solution of hemin. For the estimation of non-heme iron content in experiment and control, ferrozine reagent was added and estimated by reading the absorbance at 560nm in the presence of standard solution of Fe^{++} . The comparison of obtained heme and non-heme iron between parasites primed and non-primed erythrocyte was done to assess the consumed heme and non-heme by provided parasites.

Further, metacyclic promastigotes from culture were initially incubated with hemoglobin in separate wells at $24 \pm 1^\circ C$ and further at $4^\circ C$ to allow for attachment and internalization of hemoglobin. The parasite bound hemoglobin was treated with human anti-haemoglobin antibody at $37 \pm 1^\circ C$. The attached hemoglobin inside the flagellar pocket of parasite was observed under fluorescent microscope. Percent of hemoglobin bound to *Leishmania* was estimated by Flow Cytometry.

PBMC from healthy / patients were isolated and incubated overnight at $36 \pm 1^\circ C$ in CO_2 incubator. Macrophage was treated with *Leishmania* with or without Hb and incubated for 1hr at $36 \pm 1^\circ C$ in CO_2 incubator. Macrophages were scraped out with the help of cold trypsin. Cells were fixed with Cytotfix/ctoperm sol. Washed with Perm/wash sol. Re-suspend in PBS and treated with human anti-haemoglobin antibody-FITC. Cells were observed under fluorescent microscope. Analysis is being carried out using Flow Cytometry.

Observations:

The preliminary data showed that erythrocytes number was found 1.5 fold less in parasite primed than non-primed erythrocytes of patients and 1.1 fold less in parasite primed than non-primed erythrocytes of healthy control (Fig 1).

The hemoglobin level was found 1.2 fold less in parasite primed than non-primed erythrocytes of patients and 1.1 fold less in parasites primed than non-primed erythrocytes of healthy control (Fig 2). The heme level was 1.8 fold less in parasite primed than non-primed erythrocytes of patients and 1.1 fold less in parasites primed than non-primed erythrocytes of healthy control (Fig 3). The non-heme iron level was found almost equal fold in parasite primed than non-primed erythrocytes of patients and healthy controls respectively (Fig 4).

The data shows that parasites require low heme content yet adequate supplies to gradually release for their growth and survival whereas no clear conception has come regarding the use of non-heme iron by parasites (promastigotes).The above preliminary data may reflect the cause of anemia gradually in patient and therefore, extensive work is required.

Fig. - 1

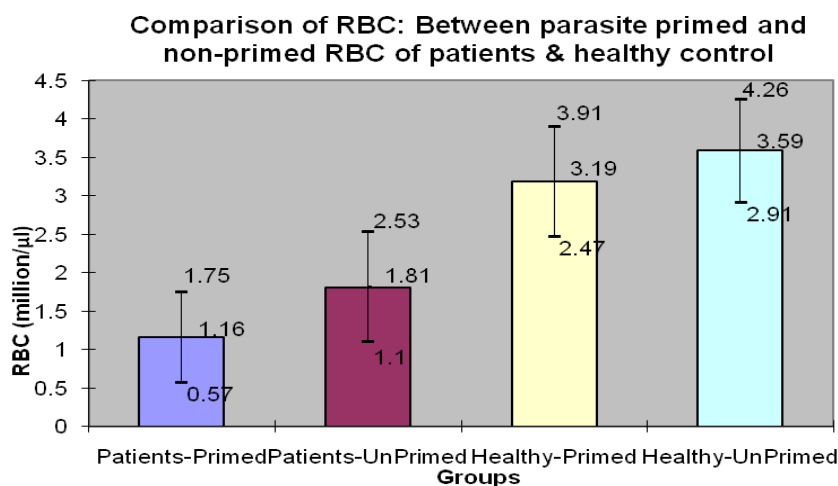


Fig. - 2

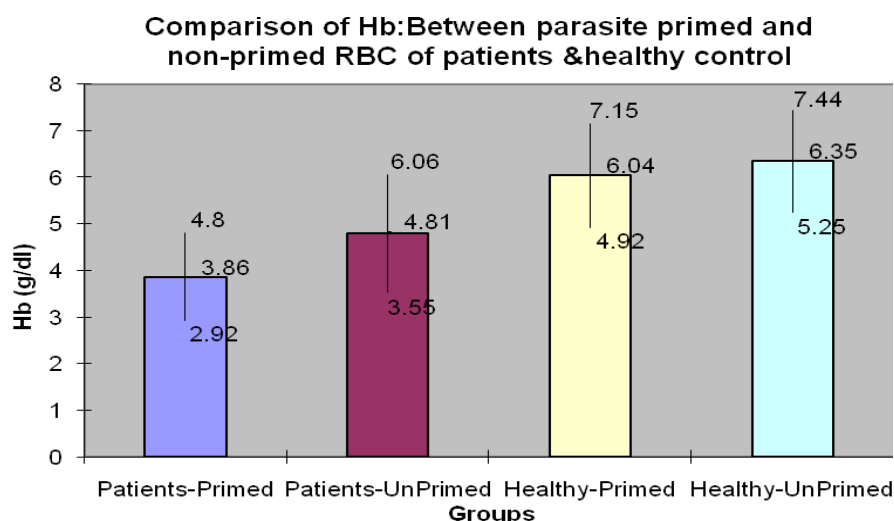


Fig. - 3

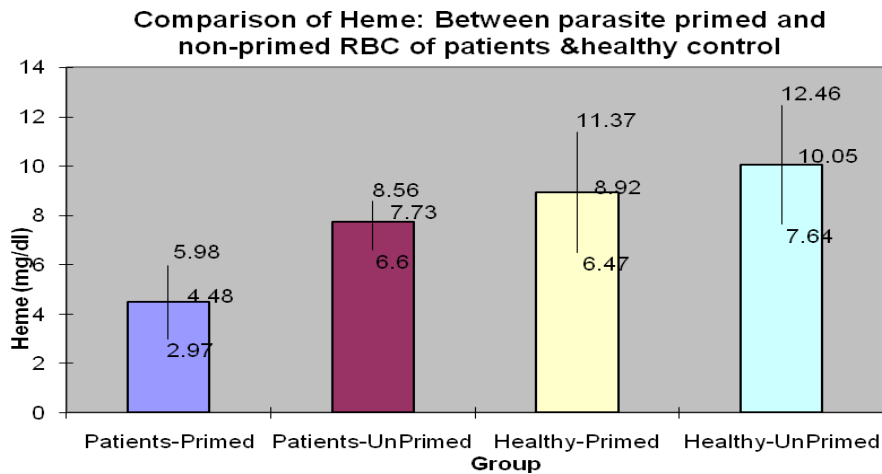
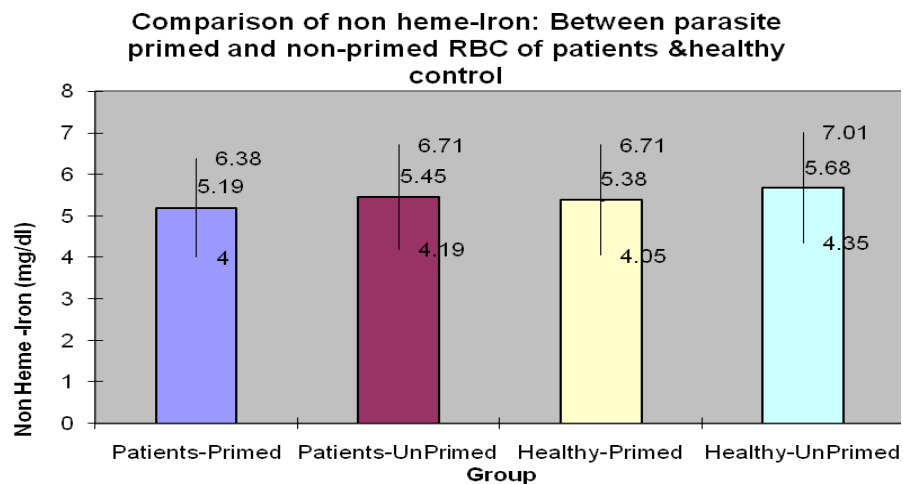


Fig. - 4



19. Study of Haemoglobinopathies in anaemia of kala-azar cases from Bihar.

N. Verma et al.

Objectives:

- To screen the anaemic cases of kala-azar and others for presence of any disorder of haemoglobin, i.e, β -thalassemia, sickle cell anaemia (HbS) etc.
- To correlate the findings with Hb%, RBC morphology, MCV, MCH, MCHC and reticulocyte count.
- To screen the cases and identify carriers of β -thalassemia and other abnormal hemoglobin for genetic counseling.

Progress:

Blood samples (about 3 ml) from 125 kala-azar patients and 50 other cases from RMRI OPD were collected in EDTA vials and the blood smears were prepared. Properly mixed blood samples were tested in Automatic Blood Cell Counter for complete blood count (CBC). Haemoglobin %, Total RBC, WBC and Platelet counts, differential counts of WBC, PCV, MCH, MCHC MCV were recorded. Stained blood smears were examined microscopically for presence of any morphological changes in RBC i.e. microcytes, macrocytic, hypochromic, hyperchromic or normochromic, target cells, any basophilic stippling, immature RBC or any other abnormality.

Blood samples of both anaemic or nonanaemic cases of kala azar and other cases were run in the 'Variant' machine to detect any abnormal haemoglobin. Percentage of Hb., HbA, HbA₂ and HbF have ben presented in the table with other haematological findings (i.e. Total WBC, RBC, MCV, MCH, MCHC and hematocrit (PCV)). Study is in progress.

Table: Value of Hb., HbF, HbA0 & HbA2 with hematological findings in kala azar and other cases. (mean, S.D., range)

Type	Age	HB%	WBC	RBC	MCV	MCH	MCHC	PCV	HBF	HBA0	HBA2
KA Cases	24..4	9.7±	5100±	3.44±	70.2	28.6±	40.3 ±	24.3±	0.28±	87.68±	3.61±
	(5- 60)	(4.7- 13.1)	(1000- 10300)	(1.72- 5.27)	(54- 91.5)	(22.5- 37.7)	(24.5- 44.3)	(11.2- 40.2)	(0.0- 1.4)	(60.9- 91.3)	(1.1- 29.8)
Non kala azar control	32.4	11.9±	8485±	4.05±	76.5±	29.9±	39.2±	30.6±	0.12±	86.3±	3.31±
	(7- 59)	(5.6- 16.1)	(2300- 16800)	1.64- 5.73)	44.2- 92.8)	(15.8- 36.2)	(35.7- 41.6)	(14.4- 42.7)	(0.0- 2.2)	(82.2- 89.4)	(1.4- 6.6)

20. Innate Immunity function in Visceral Leishmaniasis and under malnutrition.

Vikash Kumar et al.

Objectives:

Primary objective

- to define the role of innate immune response in non malnourished VL and malnourished VL patients.

Secondary objectives

- To study the expression of surface molecules of monocytes, neutrophils and NK cells.
- To study phagocytotic activity and respiratory burst activity of monocytes and neutrophils.
- To study the production and secretion of chemokines pro and anti- inflammatory cytokines of monocytes, neutrophils and NK cells.
- To analyse the mechanism of Chemokine/cytokine production/down regulation (Jak/Stat pathway) in malnourished VL patients.

Progress:

The surface expression molecules viz CD66b, CD62L, CD11b, CD69, CD119 and respiratory burst, phagocytic activity, chemokines and cytokines secretion were determined in peripheral blood of malnourished, non malnourished VL patients and healthy subjects after stimulation with SLA, LPS, PMA Io and K-562 myeloma cells. In unstimulated innate cells of VL patient CD62L expression was observed very low (479 mean values) compared to control (1253 mean value). The Mean fluorescence intensity (MFI) for CD11b before and after LPS treated was lower in VL patients (18865, 23619) compared to control (32473, 58704)). The ROS production and phagocytosis activity as means result obtained the malnourished VL patients were not significant on stimulation with SLA, LPS, PMA Io and K-562 myeloma cells. The pro and anti inflammatory cytokine viz IP-10, MIP-1alfa, IFN-gamma, IL-10, IL-8 produced by innate immune cells of malnourished VL patients after stimulation with LPS, SLA PMA Io and K-562 myeloma cells were less compared to control. CD107a (lamp-1) expression on activated NK cells in patients were also significantly low compared to control. It was observed that malnutrition has significant impact on activation of PMN, monocytes and NK Cells. The study is in progress.

21. GPI-anchored membrane proteins of *Leishmania donovani* mediated regulation of Toll-Like Receptors and costimulatory molecules on antigen presenting cells and induction of cytokines.

S. Das et al

Objectives:

- To isolate and characterize GPI-anchored membrane proteins from promastigotes of *L. donovani*.
- To study the cell proliferation and surface expression of Toll-like receptors (TLRs) in GPI anchored proteins stimulated human PBMC derived antigen presenting cells.
- To study the role of GPI anchored proteins in production of pro-inflammatory cytokines of the antigen presenting cells.
- To study the translocation of NF-kB from cytoplasm to nucleus of the APCs.
- To determine the capacity of GPI anchored proteins to induce maturation of DC by up-regulating the expression of co-stimulatory molecules.

Progress:

In the first year, GPI anchored membrane bound proteins were isolated and characterized from promastigote cultures of *Leishmania donovani*. APCs were analyzed by FACS for surface expression of TLR2, TLR4 and TLR9 after stimulation with GPI-anchored proteins. GPI-anchored protein-mediated cell proliferation was also studied by cell cycle analysis. The GPI-anchored proteins treated APCs were studied for the intracellular production of pro-inflammatory cytokines by FACS and also studied at the mRNA level. In the second year, the GPI-anchored protein mediated regulation of cytokine synthesis was studied at the cytokine release levels by ELISA. Interestingly, it was observed that GPI-anchored proteins were able to produce enough amount of IFN- γ in-vitro in healthy controls but not in VL patients. As it was previously shown that GPI-anchored proteins are potent ligands of the TLRs, we studied the responsiveness of TLRs in VL patients. Surprisingly, it was observed that TLR2 and TLR4 demonstrated reduced responsiveness on VL patients as stimulation with specific ligands like LPS and Pam3Cys did not result in any significant production of cytokines. Human control APCs showed enhanced production of IFN- γ , TNF- α and IL-12 after stimulation with GPI-anchored proteins, but no significant change was found for production of IL-10. The translocation of NF-kB was studied for understanding the

downstream signalling pathway leading to cytokine synthesis with GPI-anchored proteins. This was done with flow cytometry and it was observed that the proteins were able to translocate NF- κ B, which denotes their potential role in pro-inflammatory cytokine production.

The study is in progress to further determine the capacity of GPI-anchored proteins to induce maturation of DC by up-regulating the expression of co-stimulatory molecules like CD80, CD86 and CD40.

22. Studies on immunological changes in lymphocytes after leishmania infection.

R. Banerjee et al.

Objectives:

The overall objectives of the proposal are to study the fate of the anergic lymphocytes in infected hamster and also in patients and especially in different cell population in presence / absence of various molecules.

- To find out the mode of cell death and the pathway in the leishmania infected animal model.
- To observe the anergic lymphocyte population in patients during treatment.
- To identify the responsible cell population for the lymphocyte death.
- To identify the specific molecule for the lymphocyte death.
- To study different cell surface marker in the patients and its changes during the treatment.

Progress:

During leishmaniasis, impaired lymphoproliferation had been reported in *L. donovani* infected humans and animals. However, the fate of anergic lymphocytes is still elusive. Though mice model is widely used in VL research but the hamster model closely mimics the human active VL, so in this study, *L. donovani* infected hamster has been used to study the mechanism of lymphocyte cell death. Lymph nodes were excised from 6-7 weeks post infected hamsters and single cell suspension was analyzed for apoptotic death by phosphatidyl serine externalization, mitochondrial abnormality, caspase activity and DNA degradation. The data demonstrate that the disease progression leads to a gradual impairment of lymphocyte proliferation which can not be restored even in the presence of

concanavalin A stimulation. Fate of the anergic lymphocytes is intrinsic apoptosis as evident by depolarization of mitochondrial membrane potential, cytosolic release of cytochrome C, caspase activation and DNA fragmentation. More interestingly, TGF- β , secreted by macrophage like adherent cells, was significantly up regulated in lymph node compartment of infected hamsters. Addition of neutralizing TGF- β antibody and recombinant TGF- β showed downregulation and induction of lymphocyte apoptosis, respectively. Furthermore, it has been observed that TGF- β triggers apoptotic death of lymphocytes through the upregulation of tyrosine phosphatase activity and the use of sodium orthovanadate (NaOV, a tyrosine phosphatase inhibitor) reduces the apoptotic frequency. Thus this study clearly reports the novel involvement of tyrosine phosphatases in TGF- β induced lymphocyte apoptosis in *Leishmania* infected hamsters. Recently lymphocyte apoptosis is reported due to serine-threonine phosphatase (PP2A) activation in BALB/c mice. TGF- β signaling requires ser/thr kinases while PP2A blocks the ser/thr signaling. Thus it is very obvious question that why and how *Leishmania* induces both, the TGF- β and PP2A for the same function. To address this query and also to study in details of the signaling molecules, I finally shifted to mice model. In mice model also, the upregulation of TGF- β , T cell apoptosis and TGF- β induced PTPase activation were noticed. Additionally, I have found that TGF- β itself induces the PP2A activity. TGF- β follows the SMAD independent pathway and phosphorylates the TGF- β activated kinase (TAK)-1 molecule. It was also observed that the enhanced PTPase activity dephosphorylates the ZAP-70, the key molecule involved in the TCR signaling. On the other hand, the high PP2A activity inhibits the survival signal by dephosphorylating the ERK-1/2. The use of sodium orthovanadate (PTPase inhibitor) and okadaic acid (PP2A inhibitor) confirms the findings that TGF- β induces both the phosphatases and impairs the cellular signaling and finally causes apoptosis because the inhibitors reduce the apoptosis and also rescue the cellular signaling. This probably suggests that the use of inhibitors may restore the cell-mediated immune function of the host and thus may help in parasite elimination.

It was observed that TGF- β induces both phosphatases (PTPase and PP2A) and lymphocyte apoptosis via mitochondrial pathway. The study is concluded.

23. An analysis of the *Leishmania donovani* parasite and part played by its antigen on immunological imbalances during visceral leishmaniasis.

S. Bimal et al.

Objectives:

- To identify *Leishmania* antigens at different stages of development and their influence on Th-1 and Th-2 effectors.
- To purify the individual components of antigens of *Leishmania* from different developmental stage involved with sudden overexpressed Th-2 effector cells.
- To study the signal transduction machinery utilized by the antigens with Th-2 effector cells involved.
- To evaluate strategy to replace Th-2 signal transduction machinery by a Th-1 signal transduction shift.

Progress:

Two antigens i.e. SLA and KMP-11 were studied. KMP-11 protein cloned in pQE-30 plasmid was isolated from KMP-11 gene along with pREP-4, which were already transformed to DH5- α strain of E.Coli. Upon culture, plasmid multiplied which was induced by isopropyl 1- β -D- thiogalactoside which resulted in expression of His-tagged protein. Protein purification was done by passage of whole bacterial cell extract through a Ni-NTA affinity column. Purity of KMP-11 protein was confirmed by presence of single band only in SDS-PAGE. It was further confirmed by western transfer of protein on NC membrane and immunoblotting with mouse anti-KMP-11 antibody. This protein and SLA were presented through monocyte derived dendritic cells (CD 1a positive cells) and macrophage (CD-14 positive cells) to T-lymphocyte to study differential pattern of signalling and cytokine response.

MoDCs stimulated with KMP-11 showed much declined IL-10 production than SLA and at the same time were able to produce IL-12 pre-dominantly. In macrophages, none of such effects were seen with KMP-11 or SLA. KMP-11 led to only 1.43 fold decline in the activity pattern for smad-4 in DCs. Smad-4 analysis of KMP-11 exposed macrophages is underway. Unlike to SLA, KMP-11 priming of macrophage was more effective in lowering

down level of TGF- β than MoDC. In contrary KMP-11 helped DCs to produce huge quantity of NF-K β as compared to SLA. This effect of KMP-11 on NF-K β was observed and translated into 7.15 fold more IFN- γ produced by MoDC in KMP-11 pulsed culture. NF-K β experiment using macrophage as APC is underway. All experiments were performed on healthy cells. The study is in progress to further perform the above experiments on clinical samples of VL.

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

S. Bimal et al.

Objectives:

- To understand the CD2 deficiency in Kala-azar 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:

We evaluated the effect of combining CD2 with conventional antimonial (sb) therapy in protection in BALB/c mice infected with either drug sensitive or resistant strain of *Leishmania donovani* with 3×10^7 parasites via-intra-cardiac route. Mice were treated with anti CD2 adjunct SAG sub-cutaneously twice a week for 4 weeks. Assessment for measurement of weight, spleen size, anti-*Leishmania* antibody titre, T-cell and anti-*leishmanial* macrophage function was carried out day 0, 10, 22 and 34 post treatment. The combination therapy was shown boosting significant proportion of T-cells to express CD25 compared to SAG monotherapy. Although, the level of IFN γ was not statistically different between combination vs monotherapy ($p=0.298$) but CD2 treatment even alone significantly influenced IFN γ production than either SAG treatment ($p=0.045$) or with CD2 adjunct SAG treatment ($p=0.005$) in LD-S strain as well as in LD-R strain. The influence of CD2 adjunct treatment was also documented in anti-*leishmanial* functions in macrophages. As shown, the super-oxide generation began enhancing very early on day 10 after SAG treatment with CD2 during which SAG action was at minimum. Interestingly, the super-oxide generation ability remained intact in macrophage after treatment with immune-chemotherapy even in mice infected with *Leishmania* resistant strain. Unlike SAG treatment, treatment of SAG with CD2

also led to production of nitric oxide and TNF- α , resulting in most effective clearance of *L. donovani* from infected macrophages. Our results indicate that CD2, which can boost up a protective Th1 response, might also be beneficial to enable SAG to induce Macrophages to produce Leishmanicidal molecules and hence control the infection in clinical situation like Kala-azar. Drug resistance is the major impedance for disease control but the encouraging results obtained after infecting mice with resistant strain of the parasite strongly imply that this drug can be effective even in treating resistant cases of Kala-azar.

25. *Leishmania donovani* antigen and their influence on Natural T-regulatory Cells in immuno-suppressed VL patients.

S. K. Singh et al.

Objectives:

1. To explore the possible existence of Natural T-regulatory cells in human VL subjects.
2. To identify the possible correlation if any between *Leishmania* parasite and increased accumulation and proliferation of Natural T-regulatory cells.
3. To investigate the possibility of inactivation of *Leishmania* antigen to prevent accumulation of NTRs and hence to activate immune system to control leishmaniasis.

Progress:

Regulatory cells (adaptive and natural T regulatory cells) are reported to be a considerable source of IL-10 and TGF- β , which deactivates protective IFN- γ dominant Th1 response in many diseases. Expression of Natural T-regulatory cells (NTregs) were examined in peripheral blood mononuclear cells (PBMCs) of 20 VL cases compared to healthy subjects using florescence labeled monoclonal antibodies on Flow cytometry. This study was further extended to evaluate effect of amphotericin B therapy and accumulation NTregs in bone marrow aspirates compared to PBMCs of untreated VL subjects. Cells with phenotypic expression of CD4⁺ CD25^{hi} were found positive to Foxp3 and negative to CD127 and HLA-DR. These cells were also found positive to IL-10 and TGF- β . Up-regulated expressions of natural T regulatory cells were observed in VL patients before start of treatment compared to healthy control. There was no significant difference in the expression of ATregs in VL patients and control. It was also observed that the expression level of NTregs were higher in bone marrow aspirates (in control, bone marrow aspiration was not done). The relation of

these T-regulatory cells with immunosuppressing cytokines were also investigated and observed that the level of IL-10 and TGF- β was comparatively higher in samples with high frequency of natural CD4 T-regulatory cells.

While dealing with expression of NTregs in presence of *Leishmania* antigen an increasing trend in natural T-regulatory cells was observed in response to *in vitro* stimulation with *Leishmania*. In adaptive transfer experiments, natural CD4 T-regulatory cells from VL subject were incubated with macrophage from healthy subject and natural CD4 T-regulatory cells from healthy subject were incubated with macrophage from VL subject in presence or absence of *Leishmania* parasite. It was observed that the natural CD4 T-regulatory cells proliferated on presence of *Leishmania*. To correlate the proliferative response of natural T regulatory cells with pathogenicity of *Leishmania*, it was experimented and observed that expression level natural T-regulatory cells were significantly high in mice infected with *Leishmania* having major population of those positive to phosphatidyl serine positive. It was also observed *in vitro* studies on peripheral blood of human VL subject and found comparable. The mechanism by which natural CD4 T-regulatory cells proliferate in response to antigen is on progress.

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

N. Verma et al.

Objectives:

- 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

Specific 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 cytokine (IL-2, IFN- γ , IL-4 and IL-10) in PKDL cases and compare it with VL and control subjects.

Progress:

T-cell subsets and cytokine profile has been studied in 26 PKDL cases and 8 healthy controls. Study conducted on peripheral blood mononuclear cells of PKDL cases reflect that the absolute number of circulating CD4+ and CD8+ T cell subpopulation and surface expression during active infection in PKDL cases was reduced (mean value 655/ μ l and 499/ μ l respectively) in compare to those of healthy controls (mean value of CD4+ cells 1064/ μ l and CD8+ cell 699/ μ l). The involvement of the CD4 & CD8 T cells in the pathology of PKDL lesions will be studied in the dermal lesions. Lower expression of cytokines IFN- γ (> 1-fold) and IL-4 in CD4 T-cells of peripheral blood from PKDL cases as compared to control indicates immunosuppression in PKDL cases. The study is in progress to find out whether the PKDL Leishmania parasite is causing T-cell anergy or it is due to localized infection.

27. Protective efficacy of purified membrane antigens (Phospholipids vs Lipophosphoproteins) isolated from *Leishmania donovani* metacyclic promastigotes.

S. Bimal et al

Objectives:

- Detection of protein antigen with glycopospholipids anchors in *L. donovani* promastigotes.
- Biochemical characterization of antigen proteins lipids and phosphate.
- Assessment of protective immunity

Progress:***Immunoprotection of phospho-protein of L. donovani:***

Mice of 4 different experimental group formulations are monitored for obtaining survival rate in %. No death is reported till date in any of the experimental groups.

Anti-leishmania antibody response at different time points after immunization:

The group immunized with soluble leishmania antigen and phosphoprotein with 22 KDa protein induced a high antibody response in all 5 (100%) mice on day 7 compared to their

counterparts immunized with phospho-proteins where only 2 of 5 mice were observed with antibody titre.

Cytokine response to SLA or phosphoprotein antigen:

Increased Th-2 cells associated function in phosphoprotein immunized hosts was later observed with raised IL-10 (6.4 fold) and IL-4 (about 2.7fold) production in correspondence to about 8.57 fold more production of IFN- γ by T-cells compared to values obtained in infected unimmunised host. More enhanced values for IL-10 (24.1fold) and IL-4 (11.70 fold) were observed in lymph node. When 22 kDa protein antigen was added in the vaccine construct of phosphoprotein, notably IFN- γ was produced after 22kda addition to phosphoprotein but it was only 3.8%. Th-2 cells function was observed still promoted greatly in spleen by phosphoprotein but in spleen, IL-10 and IL-4 increased up to 1.8 fold and 2.0 fold but at this stage, IFN- γ release was observed almost undetectable. An almost similar result was observed when phosphor-protein was given along with 22kDa antigen in spleen.

It appeared that phosphoproteins studied were more related to increased pathogenesis of VL with little impact on the protective immune response.

Table: % of IFN- γ , IL-4 and IL-10 produced *ex-vivo* by lymph node cells and splenocytes from *L. donovani* infected mice before and after immunization.

Cytokine	% of cytokine produced <i>ex-vivo</i> by lymph node cells				% of cytokine produced <i>ex-vivo</i> by splenocytes			
	G-1 (Inf)	G-2 (SLA)	G-3 (PP)	G-4 (PP+ 22KDA)	G-1 (Inf)	G-2 (SLA)	G-3 (PP)	G-4 (PP+ 22KDA)
IL-4	0.8	2.70	21.60	8.2	9.4	11.40	21.6	28.35
IL-10	0.34	0.70	2.18	8.2	1.52	0.67	2.76	1.6
IFN- γ	1.00	0.60	8.57	2.2	0.6	6.90	0.6	4.5

Inf: Infected, PP: Phosphoprotein

28. Biochemical and functional characterization of Iron-sulfur cluster (ISC) assembly and cellular localization of LdIscS, LdIscU proteins in *L. donovani*.

V. Ali et al.

Objectives:

- Characterization of Iron-sulfur (Fe – S) clusters assembly biosynthesis and Fe – S proteins to understand parasite survival and multi-drug resistance mechanism.

Progress:

Leishmania genome search using NCBI/TIGR/Sanger/*Leishmania* database showed the presence of ISC system including CIA and export machinery in *L. major* and *L. infantum*, which suggested the presence of complete ISC system in the *Leishmania* parasites. Thus, genome wide analysis of Fe-S clusters machinery components is clearly showed the presence of complete ISC system which can produce Fe-S clusters in the mitochondria, cytoplasm, nucleus and their maturation as well as export. *Leishmania* parasites lack NIF and SUF systems those present in other organisms. NIF system is present in limited organisms especially in microaerophilic or anaerobic organisms.

The IscS and IscU genes were amplified using the genomic DNA isolated from the Ag83 strain of *L. donovani* using Pfu DNA polymerase. The IscU gene was PCR amplified using primers designed for recloning it in vector pGEX-4T-1. The amplified PCR product was gel purified, double digested and cloned in the aforesaid vector. These clones were screened for orientation using colony PCR and restriction digested to check the intactness of Restriction sites. We isolated the plasmids from 4 clones and used one of them to standardize the protein expression in BL-21(DE3) cells. Maximum expression in soluble fraction was obtained at 20°C, 1mM IPTG, 16 hrs induction. Recombinant IscU with N-terminal GST fusion tag was then purified using Glutathione agarose affinity column where we obtained upto 90% purity. For removing the GST-tag, the purified protein was subjected to overnight thrombin digestion in the column using 25 µl thrombin of 50 ml culture pellet (approx. 2 mg protein). Digested IscU obtained was more than 90% pure. The purified LdIscU protein collected for antibodies production in rabbit and some biochemical analysis.

LdIscS gene cloned in pGEX-4T-1 successfully showed large expression of recombinant protein in inclusion bodies. We tried to purify the protein from solubilized

inclusion bodies using different refolding methods (Sarkosyl, Triton X 100/CHAPS buffer) but unfortunately failed to get active protein due to denaturation of GST tag which prevent binding to the glutathione agarose. Again we recloned it in pCold DNA I vector which has a cspA promoter with an N-terminal His-tag to get the protein in soluble fraction but all the proteins were found in the inclusion bodies again. The protein expression was good so we have tried to purify the protein from inclusion bodies. We have purified IscS protein successfully upto 95% pure using denaturing Ni-NTA affinity column chromatography. We have got sufficient amount of protein in denatured condition for antibodies production. Refolding of purified denatured IscS was attempted using various methods but the yield of refolded protein was not more than 40 µg/ml. These refolded proteins lacked cysteine desulfurase activity. We have again cloned in pET-28a vector and protein is expressed in inclusion bodies. Currently, we are trying to devise a proper refolding procedure for IscS protein. Also we are raising antibodies against recombinant IscS and IscU proteins in rabbit for immunoblot studies.

29. Analysis of Isd11 and frataxin interaction and their roles in Fe-S cluster machinery in *Leishmania donovani*.

V. Ali et al.

Objectives:

- Biochemical and functional characterization of Frataxin and Isd11 homologues of *L. donovani*
- To find out interaction between Frataxin & IscU and Isd11 & IscS
- Sub-cellular localization of Isd11, frataxin and their functional roles in *L. donovani*

Progress:

Leishmania genome, searched using NCBI/TIGR/Sanger/*Leishmania* database, showed the presence of frataxin and Isd11 homologues in both *L. major* and *L. infantum* and suggested the presence of ISC system for Fe-S clusters biogenesis in *Leishmania* parasite. The Frataxin (fra) and Isd11 genes were amplified using the genomic DNA isolated from the Ag83 strain of *L. donovani* using Taq DNA polymerase (Roche), Pfu-DNA polymerase, respectively. Amplified product was purified by gel extraction kit (Qiagen). The amplified gene of frataxin homologue was digested using restriction digestion enzymes (BamH1 and

XhoI). Similarly the pGEX-4T-1 vector possesses N-terminal GST residue was also digested and both were purified and ligated using the Qiagen PCR cloning kit. We got positive clones which possess frataxin gene in correct orientation after confirming with Colony PCR and restriction digestion of the constructs (plasmids). The Fra construct was transformed in the competent BL-21 (DE3) *E. coli* cells and currently standardization of the conditions for getting expression at optimum levels by varying temperature, time and concentration of inducer is in process. Cloning of Isd11 gene in the plasmid containing histidine tag is also under progress.

30. Mechanism of Amphotericin B Resistance in *Leishmania donovani* parasites

N. Nandi et al.

Aims and Objectives:

The overall aim of the proposed project is to study the resistant mechanism conferred by the drug resistant *Leishmania* parasites against Amphotericin B and probable difference in the resistant and sensitive strains in sterol composition and content, drug efflux mechanisms and ROS mediated cell signaling pathways in conferring resistance. This study will help in identifying the key factors associated in mediating drug resistance which may eventually lead towards the finding of newer targets for future rational chemotherapeutic drug designing.

Specific Objectives:

- To select AmB resistant and sensitive parasites.
- To analyse the difference in the content and composition of the free sterols and fatty acids in resistant and sensitive strains
- To study the uptake of AmB in sensitive and resistant Parasites with time
- To study the efflux of AmB from sensitive and resistant Parasites with time and probable involvement of ABC Transporters in drug efflux if any
- To study the difference in thiol levels in drug sensitive and resistant parasites
- To study the difference in the expression level of Trypanothione dependent peroxidase, Na⁺-K⁺ ATPase, Trypanothione reductase, γ -glutamyl cysteine synthetase and Ornithine Decarboxylase in the resistant and sensitive parasites with time

- To study the generation of ROS in both drug resistant and sensitive parasite with time and to find out the possible difference in ROS mediated pathway in both resistant and sensitive parasite if any

Progress:

In the first year of study, Amphotericin B resistant and sensitive Strains were selected by in vitro and ex vivo Drug Sensitivity Assay. Lower uptake and higher efflux rate of AmB was observed in resistant promastigotes compared to sensitive. Relative expression level of ABC Transporters (higher mRNA level of MDR1 and approximately similar mRNA level of PgPA) were assessed using semi quantitative RT-PCR. It was observed that there was higher accumulation of ROS in sensitive strain as compared to resistant and higher expression level of Thiol Metabolic Pathway genes involved in ROS detoxification in resistant promastigotes as compared to sensitive. The study was further continued with the following findings:

Total Intracellular thiol level

The total intracellular reduced thiol content was found to be ~2 fold higher in resistant strain compared to sensitive.

Comparative analysis of the membrane fluidity and Intracellular K⁺ level

The emission anisotropy value of AmB-resistant *Leishmania* promastigotes was three times lower indicating a higher fluidity for the membrane of the resistant strain compared to sensitive. The AmB treated sensitive promastigotes demonstrated ~2.2 fold decrease in the intracellular K⁺ level indicating higher membrane depolarisation compared to sensitive.

Lower expression level of SCMT gene

SCMT (S-Adenosyl-L-Methionine:C-24- Δ -Sterol Methyltransferase) is an important enzyme in sterol biosynthetic pathway as it performs C-24 transmethylation which is a key step to produce ergosterol. It was found that SCMT is very poorly expressed in the AmB Resistant parasites compared to the sensitive indicating an altered sterol biosynthesis in the resistant strain.

Reversion of AmB resistance Property by inhibitors of ABC transporter and Thiol Metabolic Pathway

It was observed that verapamil (an inhibitor of MDR1) reverses the AmB resistance phenotype partially which has been demonstrated by partial inhibition of AmB efflux (~2 fold) and decreased LD50 value (~2 fold) for the resistant parasites. Thiol metabolic pathway inhibitors (BSO and DFMO) also reverses the resistant phenotype partially to sensitive which has been demonstrated by decreased intracellular thiol content ~1.4 fold and LD50 value ~1.8 fold for the resistant strain. The co-inhibition effect on the resistant strain with both thiol metabolic pathway inhibitor and ABC transporter inhibitor were also examined. It was found that co-inhibition has more potent effect compared to inhibiting with either ABC Transporter inhibitor or thiol metabolic pathway inhibitor in reversing the resistant property of the resistant strain as demonstrated by ~2.5 fold decrease in the LD50. This may probably indicate towards a synergistic involvement of both drug efflux and ROS scavenging machinery in conferring AmB resistance.

The study is in progress to observe difference in the content and composition of the free sterols and fatty acids in resistant and sensitive strains and functional characterization of few of the important genes related to AmB resistance.

31. Study of Trypanothione metabolism and associated pathways in *Leishmania donovani*: cloning, biochemical characterization and physiological significance of trypanothione synthetase and trypanothione reductase.

V. Ali et al.

Objectives:

- Characterization of Trypanothione metabolism of *L. donovani* to understand its role in multi-drug resistance mechanism.

Progress:

The ability of *Leishmania* amastigotes to survive within the drastic environment conditions and the oxidative stress in the phagolysosomes of mammalian macrophages and resistance to drugs like antimonials (SAG) is thought to be due to Trypanothione, the central molecule of the oxidative stress relieving redox system in the parasites. So the detailed study

of the Trypanothione system will increase our understanding of the intracellular survival of the parasites under stressed conditions and in presence of drug i.e. drug unresponsiveness.

TryS and TryR genes were amplified using the genomic DNA isolated from Ag83 strain of *L. donovani* using Pfu- DNA polymerase (Fermantas). These products were digested using restriction digestion enzymes Bam HI and Nde1 for TryS and BamH1 and Pst1 for TryR, similarly the vector also digested and were purified and ligated using the Qiagen PCR cloning kits. We have got many positive clones which possess TryS and TryR genes in correct orientation. The TryS gene is cloned successfully in pET-15b vector containing N-terminal His-tag to purify fusion protein with Ni-NTA affinity chromatography. Similarly the TryR gene is cloned in pCold vector having N-terminal His-tag. These clones were transformed in *E. coli* BL-21(DE3) competent cells and the expression of protein was optimized by varying temperature, time, and concentration of IPTG. We have found that the TryS protein was expressed maximum in insoluble fraction and few in soluble fraction at 30 °C, with 1.0 mM IPTG for 5-6 hrs. We have tried to reduced temperature and IPTG concentration to get maximum protein in soluble fraction. We have found maximum expression of LdTryS at 20 °C and 0.4 mM IPTG for 24 hours. We have purified recombinant LdTryS protein up to homogeneity with Ni-NTA affinity column chromatography. Large amount of protein will be purified for antibodies production and biochemical analysis. We are still analyzing the expression of TryR cloned in pCold DNA1 vector. It is found that protein was expressed very low amount in inclusion bodies.

32. Studies on genomic diversity in *Leishmania donovani* using genomic DNA microarray.

Ashish Kumar, S. Das et al.

Objectives:

Primary objective

- To work on the analysis of the genome wide diversity of the causative agent, *Leishmania donovani*, with a view to understand the genetic basis of the phenomenon such as drug resistance and infectivity that are of importance in the management of the disease.

Secondary objectives

- To construct the shot gun genomic DNA library of *L. donovani*

- To sequence the gene(s) that are differentially expressed and responsible for drug resistance and pathogenicity in leishmaniasis
- To study the functions of the important genes involved in drug resistance and pathogenesis

Progress:

Hybridization of the amastigotes derived from lesion, macrophage and axenic compared to promastigotes showed more than 2 fold reproducible upregulation in 120 arrayed clones (1.2%) in lesion-derived amastigotes, 93 (0.9%) in macrophage derived and 56 (0.5%) in axenic amastigotes than promastigote. The cluster analysis of three experiments identified 70 differentially expressed genes in the different forms of amastigotes. Genes with differential mRNA levels in amastigote stage included genes encoding enzymes involved in metabolism and which may be required for survival of amastigotes in macrophages and thereby maintaining the pathogenesis. The stage-specific proteins generally fall into five groups of functionally related proteins. These functional categories are: (i) stress response and survival (ii) cytoskeleton and cell membrane; (iii) amino acid metabolism; and (iv) cell cycle and proliferation. Among them 20 S Proteasome beta 6-subunit and Amastin like protein had been functionally characterized.

Selection of Amphotericin B Resistant and Sensitive Strain

To select true AmB (Amphotericin B) resistant and sensitive strain of *Leishmania donovani* and to determine their ED₅₀, clinical isolates were obtained from AmB responder and non responder VL patients treated in the indoor ward facility of Rajendra Memorial Research Institute of Medical Sciences, Patna and their growth kinetics as well as in-vitro and ex-vivo drug sensitivity assay was performed. The ED₅₀ for the resistant and sensitive promastigote is 0.375µg/ml and 0.125µg/ml, which for the axenic amastigote is 0.750µg/ml and 0.225µg/ml. The results also showed that the ED₅₀ of AmB for the resistant parasite is almost 3 folds higher in comparison to the sensitive parasite. These results confirmed that the resistant and sensitive clinical isolates obtained for clinically diagnosed VL cases are true resistant and sensitive strains.

Differential gene expression in Amphotericin B resistant and sensitive strains

Hybridization of drug resistant and drug sensitive strains showed 41 clones upregulated in the drug resistant strains with >3 fold upregulation. The most important genes found to be upregulated in the resistant parasite are involved in peroxide elimination and drug sequestration/efflux machinery.

Studies related to functional characterization of few important genes responsible for AmB resistance is under progress.

33. Studies on differential proteomic responses of *Leishmania donovani* on exposure to nitrosative and oxidative stress.

A. H. Sardar, P. Das et al.

Objectives:

- To identify the proteins that are differentially expressed in *Leishmania* sp. exposed to sub-lethal doses of oxidative and nitrosative stress with respect to wild type parasites (without any stress) using Differential Proteomics approach.

Specific objectives:

- To investigate the differential proteomic response of *Leishmania* under oxidative stress and nitrosative stress.
- Identification of protein molecules responsible for oxidative and nitrosative stress tolerance response.

Progress:

Selection of sub-lethal dose for oxidative and nitrosative stress in-vitro:

The optimal exposure dose for oxidative as well as nitrosative stress was observed through MTT assay using different concentration of menadione (oxidative radical donor) like 2 uM, 2.5 uM, 5 uM, 7.5 uM, 10 uM and it was found to be 5 uM at 18 hours (about 70 % cell viability) and 300 uM at 18 hours (about 50 % cell viability) respectively. When menadione and SNAP used together then the optimal exposure dose decreased to 2.5 uM of menadione and 250 uM of SNAP at 15 hours (about 62 % cell viability).

Selection of optimal exposure time for Oxidative and nitrosative stress through Antioxidant assay:

The optimal exposure time for oxidative and nitrosative stress was observed through antioxidant assay at different time from 3 h to 48 h at 3 hours interval and it was found that 18 hours when menadione or SNAP used individually however when menadione and SNAP used together then the optimal exposure time decreased to 15 hours.

Measurement of ROS and RNS level:

After exposure with the optimized dose of menadione and SNAP, the ROS and RNS level were analyzed spectrophotometrically. It was found that the level of ROS and RNS were increased 13 fold and 4 fold respectively compared to control.

Quencher study of ROS and RNS level:

To study the level of ROS and RNS in oxidative and nitrosative stressed cell, the quencher study was conducted. It was found that the level of ROS and RNS were decreased to normal level i.e similar to control after addition of quencher (trolox for ROS and Mn-TBAP for RNS).

Two dimensional electrophoresis for the differential proteomics study under oxidative and nitrosative stress:

2-D gel electrophoresis of the total protein isolated from stressed and normal parasite was performed and the better resolved portions of the gel images were analyzed. It was found that three protein spots were newly expressed when three were up-regulated and two were down regulated in stressed condition compared to normal.

Primer designing and PCR optimization for Semi-Quantitative RT-PCR & Real-Time PCR:

The primers for five genes related to stress response were designed from *Leishmania infantum* genome database and PCR amplification both from genomic DNA and cDNA have been done.

Optimization of iTRAQ labeling:

Optimization for the iTRAQ labeling from the control sample has been done.

The study is in progress to check the expression level of five genes Semiquantitative and to carry out differential proteomic study by using iTRAQ labeling and subsequent

LC/MS/MS. The differentially expressed protein molecules is to be identified by MASCOT search engine from *Leishmania infantum* protein database and then functionality of few of the important genes involved in stress will be studied.

34. Evaluation of pathogenesis in visceral leishmaniasis, part played by *Leishmania donovani* Vs vector salivary gland homogenate (SGH).

S. Narayan et al.

Objectives:

- To evaluate *in vitro* infection and immunity in mononuclear cells stimulated by SGH primed killed parasites antigen, SGH alone and killed parasite antigen alone
- To evaluate *in vitro* infection and immunity in non-stimulated mononuclear cells and parasitized by SGH primed live parasites and live parasites alone

Progress:

The late log phase of promastigotes (10^8 /ml in culture) was used for preparation of soluble *leishmania* antigen (SLA) stock. The salivary gland (20 pairs/ml in PBS) of sand flies (*P. argentipes*) was used for preparation of vector salivary gland homogenate (SGH) by six cycle of freezing thawing technique. Initially, SGH concentrations of different pairs of salivary glands (1, 2, 4, 8, 10, 20 pairs/ml) were used for standardization of optimum activation of 10^6 mononuclear cells/ml and finally the standardized concentrations of SGH with SLA of different quantum of killed promastigotes (1×10^3 , 2×10^3 , 3×10^3 /ml) was used for standardization of optimum activation of 10^6 mononuclear cells.

In one set (A) of experiment, harvested mononuclear cells were stimulated with SGH prepared by different pairs of salivary glands as test and no stimulation as control. Prior to incubation, PMA (1ng/ml), Ionomycin ($2 \mu\text{M}$ /ml) and golgi stop ($0.7 \mu\text{g}$ /ml) were added to culture. The CD_4^+ cells were stained with Per cP labeled anti- CD_4 monoclonal antibodies. The intracellular cytokines IFN- γ and IL-4 of CD_4 cells were stained by PE and APC labeled anti- human monoclonal IFN- γ and APC antibodies respectively. Iso-type control and compensation were included in all the assays. Data were acquired by using FACS calibur and analyzed by using cell quest software (Becton Dickinson, SanJose). In other set (B) of experiment the mononuclear cells were stimulated by the standardized concentration of SGH vs SLA of different number of parasites and repeated the experiment as above.

Observations:

Almost SGH concentration of about one pair of salivary glands showed optimum cells activation. The ratio of SGH prepared by one pair of salivary glands and SLA of about 2000 killed parasites showed the maximum cells activation. Evaluation of *in vitro* infection and immunity in non-stimulated mononuclear cells and parasitized by SGH primed live parasites and live parasites alone is under progress.

Bioinformatics

35. Comparative molecular modeling of various important proteins of different *Leishmania* strains and ligand-protein interaction study of different anti-leishmanial drugs.

G.C. Sahoo et al.

Objectives:

- Molecular modeling of different important proteins of *Leishmania donovani* using different algorithms i.e. homology modeling, ab initio prediction and threading.
- Compare the structures of important proteins of *L. donovani* with those of other *Leishmania* strains.
- Ligand-protein interaction study of different anti-leishmanial drugs e.g. miltefosine, paromomycin.

Progress:

Structural models of six different proteins of various *Leishmania* strains have been developed. Each protein has been tested for its ligand protein interaction with diverse sets of ligands (antileishmanial drugs, drugs / ligands acting on template protein, other ligands which may have interaction from literature) by computational methods using ligand protein interaction tools of discovery studio and GOLD software. In some cases structure of the protein is available from PDB, which is taken for this study to know the presence of interaction with already reported antileishmanial drug candidates and other predicted antileishmanial ligands. Some of other proteins of which structural model has been constructed are Enolase, Serine Hydroxymethyltransferase (SHMT), HGPRT, pyrroline-5-carboxylate reductase. Near about nine hundred compounds or their analogues have been

studied to know their ligand protein interactions with seven different important proteins (pyrroline-5-carboxylate reductase, actin, transcription initiation factor- like protein, major surface protease) of *Leishmania*.

Modeling of other important proteins of *Leishmania donovani* and their interaction with various ligands are going on. The variation in sequences(which aren't available in the NCBI database) of different important proteins are being carried out by RNA extraction, amplification of the gene by PCR and automated sequencing of the gene with specific primers.

36. Development of novel algorithm to find microsatellites in *Leishmania* genome and its database.

M.R. Dikhit et al.

Objectives:

- To develop an algorithm to find out microsatellites in *Leishmania* genome which may be used as molecular marker in different fields like genome characterization, Mapping, phylogeny and evolutionary biology.
- To develop a curated and integrated web-based relational database providing centralized access to publicly available *Leishmania* microsatellites.

Progress:

LeishMICROSATdb database of microsatellite repeats in the genome of the *Leishmania* species for which whole genome sequencing is available has been developed to make available the whole sequence data in public domain. These whole genome sequences are assembled as chromosomes. *LeishMICROSATdb* contains di to hexa nucleotide repeats of three species of *Leishmania* *L. major*, *L. infantum* and *L. braziliensis*. Precise need based microsatellites data retrieval is possible using different input parameters like microsatellite type (simple perfect), repeat unit length (mono- to hexa-nucleotide), repeat number, microsatellite length and chromosomal location in the genome. Furthermore, information about clustering of different microsatellites in the genome can also be retrieved to facilitate primer designing for PCR amplification of any desired microsatellite locus, 200 bp upstream and downstream sequences to the desired microsatellite repeat are provided.

As the developed database provides good quality data, it was thought to further explore the possibility to find out polymorphic microsatellite and to predict the genetic

distance based on the repeats for which the study may be continued for another two years.

37. Development of a database of *Leishmania* species to find the cause of changing functional family based on amino acid composition.

M. R. Dikhit et al.

Objectives:

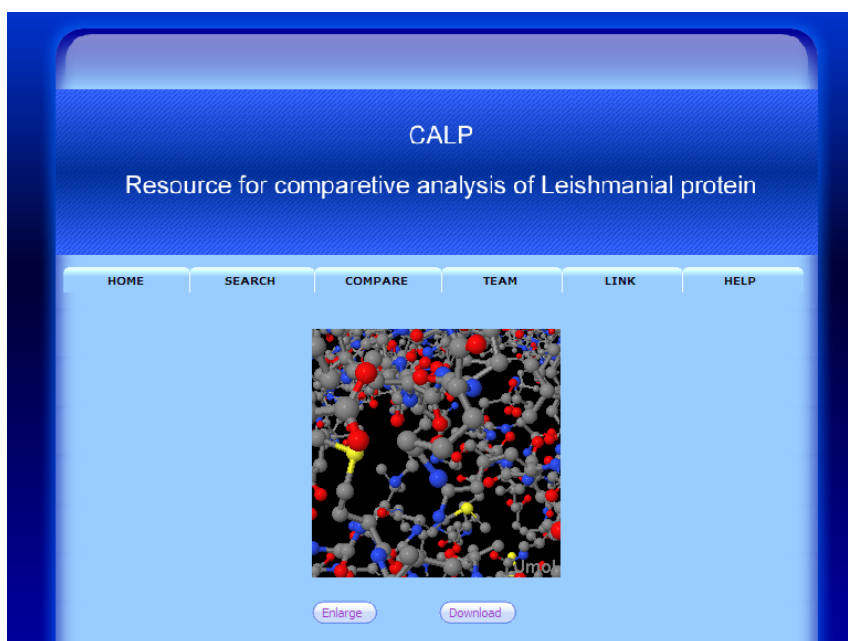
- Database of leishmania species which display the evolutionary relationship /distance related to their predicted functional family.
- Development of algorithm for constructing Pair wise global alignment.

Progress:

Identification of diverse protein functions may facilitates a mechanistic understanding of different *leishmania* proteins and opens novel means for drug development. Nearly 25 important proteins of each species have taken into consideration in the present study. Our study from SVMProt suggests that the proteins of different strains having Lyases - Carbon-Oxygen Lyases , Actin binding, All DNA-binding, Hydrolases - Acting on Ester Bonds, Magnesium-binding, Calcium-binding, Copper-binding, Metal-binding, DNA repair, Zinc-binding, Transmembrane and All lipid-binding group of functional family. But most of the proteins commonly belong to All lipid-binding proteins, Zinc-binding and Metal-binding functional family. It was analyzed that most of the *homologous* protein sequences belong to same functional group. But change in amino acid composition may affect the functional properties of the proteins. For example the analysis of RAD51 protein suggests that mutation of RAD51 protein of *L.brazelensis* may change the availability of some functional groups. From multiple sequences alignment of RAD51 protein of *L.brazelensis*, it was analyzed that the mutation of glycine to threonine , arginine to glutamine, serine to valine , valine to methionine, alanine to cysteine , glutamic acid to valine, proline to phenylalanine, glutamine to proline, serine to glycine , aspartic acid to glycine, methionine to valine, cysteine to tyrosine and serine to alanine at different position may lack the availability of Aptamer-binding protein, Outer membrane and RNA-binding functional family and availability of Lyases - Carbon-Oxygen Lyases, Actin binding, All lipid-binding proteins group of functional family. Mutation of arginine to glutamine at 41 position and valine to isoleucine at 321 position may increase the availability of Oxidoreductases - Acting on the CH-CH group

of donors and Manganese-binding functional family. In *Leishmania donovani* the insertion of proline at 43rd position and mutation of glutamic acid to aspartic acid may increase the presence of DNA recombination and mRNA slicing functional family. Similarly, the user can analyze other proteins available to the database.

Fig.: Screenshot of the database



38. Computer Aided Drug Design: Structure determination of Elongation Factor-1 α in *Leishmania donovani* by molecular modeling and NMR spectroscopy, targeting through QSAR and pharmacophore analysis

Mukta Rani et al.

Objectives:

- Homology modeling of Elongation Factor-1 α in *Leishmania donovani*.
- Crystal structure determination of Elongation Factor-1 α protein of *Leishmania donovani* through Nuclear Magnetic Resonance (NMR) Spectroscopy.
- Docking analysis of various ligands against EF-1 α through Ligandfit (DS 2.1), GOLD 4.1 and Flex X.
- Prediction of the drug candidate that can inhibit activity of EF-1 α by pharmacophore mapping and QSAR analysis.

Progress:

After discussion with expert, as suggested by SAC, the project was redesigned and the title, objective and methodology were modified.

In the present form of study it was considered that Elongation Factor-1 alpha (EF1- α) protein, an essential component of the eukaryotic translational apparatus, is a GTP-binding protein that catalyses the binding of aminoacyl-transfer RNAs to the ribosome. Computational models of EF1- α have been constructed by using (template PDB id: 2b7C_A) the Yeast guanine nucleotide exchange factor eEF1B alpha K205A mutant in complex with eEF1A through MODELLER software 9v7 and Discovery Studio 2.1. Multiple alignment of amino acid sequences of EF1- α protein of different species showed all were very close to each other ranging from 77-92 sequence identity score. A hairpin of 12 amino acids was modelled that was unique to the human EF1- α protein but in *Leishmania*, it was missing and so it had significant opportunity to design novel, small molecule inhibitors that bind specifically to the region. The quality of the modelled protein was further validated and evaluated with Ramachandran's plot calculations using PROCHECK to obtain a stable structure. Stereo chemical evaluation of backbone Psi and Phi dihedral angles of modelled EF1- α protein was revealed in different percentages i.e. 89%, 38% and 3% residues falling within the most favoured regions, additionally allowed regions and generously allowed regions and few residues were in disallowed region of Ramachandran's plot respectively. The resulting model has the correct stereochemistry as gauged from the Ramachandran plot and good three- dimensional (3-D) structure compatibility as assessed by DOPE score (207.58). The 3-D structure of EF1- α protein has one chain, 20 β - sheets, and 8 alpha helices. Potential ligand binding sites (LBSs) in EF1- α protein have been identified using Pocket Finder program.

Overall in the present study, the active site architecture and certain key residues responsible for inhibitor binding were identified, which provided insights for the design of novel inhibitors of parasitic leishmaniasis. The study is proposed to be continue for application of ADMET test, Cytotoxicity test and Protein-ligands interaction study by using in vitro techniques.

Vector Biology & Control

39. Identification of sibling species of *Phlebotomies argentipes* population in Bihar.

D.S. Dinesh et al.

Objectives:

- To find out variations among population of *P. argentipes* responsible for transmission of the disease.

Progress:

P. argentipes were collected from the indoor resting sites early in the morning using aspirator and flash light followed by the overnight collection of sandflies installing CDC light trap inside humandwellings and cattlesheds selected in three villages named Rampur Singhara (Vaishali), Rukhai (Nalanda) and Tekuna (Gaya). In total, 108 female *P. argentipes* were selected randomly to study the detail morphology. Variation in morphology, behavior, and distribution suggests it to be a complex of sibling species. The putative complex is composed of the members *Phlebotomus argentipes* sensu stricto, *Phlebotomus annandalei* Sinton 1923 status revived and *Phlebotomies glaucus* Mitra & Roy 1953 new status with the per cent distribution of 27.8, 52.8 and 19.4 respectively. Adult females of *P. argentipes* s.s. differ from those of other members of the complex based on the following morphological characters: length of ascoid <0.5 x that of flagellomere AII; pharyngeal armature occupying one third of its total length; thorax and abdomen grayish brown; tarsi silvery white; wing index 2.0 and overlap 0.2. Females of *P. annandalei* differed markedly from the other two species in the following mor-phological characters: body light brown, pleura and legs lemon yellow or light brown, wing index <1.5 and its overlap >2.5 , ascoid <0.4 x length of flagellomere II, pharyngeal armature depth 0.2 x its length, base of common spermathecal duct length 0.09 mmlong, 0.8 x length of spermathecal body. Ilango has found *P. glucus* females differentiated from those of the other two species by the following morphological characters: body gray or grayish blue but tarsi not silvery white, wing index <2.0 and its overlap 0. 15, ascoid >0.7 X flagellomere II, pharyngeal armature depth 0.25 x its length, base of common spermathecal duct. In our case ascoid <0.6 x flagellomere was found. Rest

characters were similar. The taxonomic characters of wing, mouth parts and spermathecae were considered and found relevant.

Successful clonal cross mating was done between the *P. argentipes* collected from both endemic and non endemic areas. The progeny was continuing their life cycle in F2 generations. Efforts were made to scrutinize the all three types but mass rearing could not be done successfully as it is difficult to get scrutinizing in alive condition. It was done after death followed by egg laying in individual pot. The study is completed.

40. Determination of infection of *Leishmania donovani* among *Phlebotomies argentipes* populations in different endemic areas of Bihar.

D.S. Dinesh et al.

Objectives:

- To determine the relative abundance of *P. argentipes* and infectivity rate in areas of low and high transmission zone of Kala-azar.

Progress:

Sandflies were collected seasonally from all three types of endemic areas like highly affected Rampur village of Vaishali district, moderately affected Rukai village of Nalanda district and non-endemic Tekuna village of Gaya. The density of sandflies was found changed with seasonality as reported earlier but the reported peak density in early summer was replaced by the rainy season in all three areas. This might be due to the effect of global warming. The temperature reached at about 35-40⁰C with 50-60% relative humidity in the early summer period i.e. unfavourable conditions for sandflies. However, the rain was mild and provided congenial environmental conditions for flourishing the sandfly population with relatively lower temperature between 30-35⁰C and high Relative Humidity 70-100%. *P. argentipes* were found along with *Sergentomyia* sp. in Vaishali and Gaya districts. However, *P. argentipes*, *P. papatasi* and *Sergentomyia* sp. were found in Nalanda district. Amongst the study area, only VL cases were found appearing in the Vaishali district, where as only PKDL cases were reported from Nalanda district. The density was almost similar in all districts. In total 250 gravid/ unfed *P. argentipes* were dissected in the laboratory under

aseptic conditions. None was found positive for the flagellate during microscopy. Blood meal analysis indicates preference of cattle in each district.

The study may be further explored at PHC level to see the biological behavior for preference of indoor vs out door resting/breeding habitat of sandflies along with the preventive measures against biting of sandflies to be under taken by villagers and the control measures by Govt. agency.

41. Study of host preference and behavioural changes in *Phlebotomus argentipes* in DDT sprayed and unsprayed areas of Bihar.

V. Kumar et al.

Objectives:

- To find out the effect of spraying on the behavior of sandfly.
- To find out the blood meal index in the sprayed and unsprayed areas.

Progress:

The study was further continued in the identified study area to study possible behavioural change in sandfly. Indoor and outdoor sandflies collection was made using aspirator in IRS sprayed and non-sprayed area. In both areas, sandfly was found to be endophilic as well as exophilic in nature.

Prior to conduct blood meal analysis of the sandflies, dot-ELISA test for blood meal analysis was standardized. A total of 168 female freshly fed sand flies (54 from IRS area Vaishali and 114 from non-IRS area Gaya) were collected and their blood was squeezed on filter paper and preserved in deep freeze for further analysis. Blood meal analysis revealed human, bovine and goat as host preference. Blood meal analysis were also performed on different time intervals like 2 hrs, 4 hrs, 12 hrs, 24 hrs and 48 hrs and the result revealed throughout persistent of positivity.

Figure: Standardization of dot-ELISA for blood meal analysis of different host

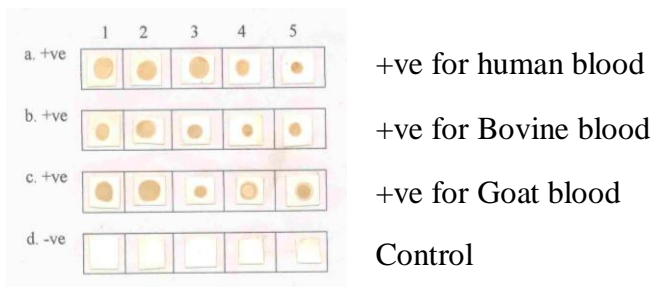


Table: Blood meal analysis of *P.argentipes* collected from endemic and non-endemic area of Bihar 2009-10.

<i>P.argentipes</i> collected from endemic area							
Biotopes	Sandfly collected	Human (+)	Bovine (+)	Goat (+)	Rat (+)	Rabbit (+)	Negative/Unknown
Human dwellings	12	8 (66.6)	2 (16.7)	0	0	0	2 (16.7)
Cattle shed	23	1 (4.4)	20 (86.9)	0	0	0	2 (8.7)
Mixed dwellings	19	3 (15.7)	14 (73.7)	1 (5.3)	0	0	1 (5.3)
Total	54	12 (22.2)	36 (66.7)	1 (1.8)	0	0	5 (9.3)
<i>P.argentipes</i> collected from endemic area							
Human dwellings	26	19 (73.0)	5 (19.3)	0	0	0	2 (7.7)
Cattle shed	47	3 (6.4)	40 (85.2)	0	0	0	3 (6.4)
Mixed dwellings	41	6 (14.6)	33 (80.6)	1 (2.4)	0	0	1 (2.4)
Total	114	28 (24.6)	78 (68.4)	2 (1.7)	0	0	6 (5.3)

42. Developing a systematic key for identification of immature stages of sand flies.

S. Kesari et al.

Objectives:

- To construct a taxonomic key to identify the immature stages of sand flies such as *P. argentipes*, *P. papatasi*, *Sergentomyia babu* etc.

Progress:

Sandflies were collected from the month of January to October, 2010 from Vaishali district (endemic site) and from the month of March to September, 2010 from Gaya district (non-endemic site) of Bihar. After rearing the sandflies in the insectorium, no change has been found among the larval stages of *P. argentipes* in their number of segments, caudle bristles, segment hairs as well as head region. Body structure of larval stages is mostly same.

Table: Development of larval Stages of *Phlebotomous argentipes* in endemic and non-endemic district

Month	Larval stages of <i>P. Argentipes</i>							
	Endemic district – Vaishali				Non-endemic district – Gaya			
	1 st	2 nd	3 rd	4 th	1 st	2 nd	3 rd	4 th
January	62	54	41	37	-	-	-	-
February	54	36	30	28	-	-	-	-
March	70	68	48	50	22	18	16	16
April	46	76	68	56	24	20	18	20
May	64	46	37	30	20	21	20	14
June	56	50	42	34	18	34	33	28
July	64	52	42	40	30	39	36	33
August	52	42	36	36	34	35	32	38
September	44	32	30	32	30	28	26	24
October	28	34	34	34	-	-	-	-
Total	540	490	408	377	188	195	181	173

Larval stages of *P. papatasi* and *Sergentomyia babu babu* is to be observed. The study is under progress.

43. Control of Indian kala-azar by genetic changing of symbiotic bacteria of the vector, *P. argentipes*.

D.S. Dinesh et al.

Objectives:

- To find out the symbiotic bacteria inside the gut of andflies and modify it genetically to make the strain refractory to the parasite.

Progress:

The work is being conducted with the collaboration of Dr. Ravi Durvasula and Dr. Kashi Nath from New Mexico. They identified the bacterial fauna at molecular level and also developed the paratransgenic bacteria to make the *P. argentipes* refractory for development of parasite in the gut with our specimens and also worked in our lab. However, sandflies were collected from the study villages and dissected under aseptic conditions under bio-safety hood. The laboratory bred sandflies were also dissected for the same purpose. The lysates of gut are preserved in -20°C keeping in microcentrifuge vial with PBS for further experiment of molecular characterization of bacterial fauna.

44. Remote Sensing and GIS: Tools for the prediction of epidemic for the intervention measures.

G.S. Bhunia et al.

Objectives:

- To identify favourable areas for breeding of vector through Remote sensing and GIS
- To identify appropriate environmental health control measures
- To demonstrate how remote sensing data acquired at various scales and spectral resolutions can be used to study spatial distribution of infectious disease pattern.

Progress:

A point data set was generated based on disease incidence report from 2000 to 2008. By calculating the X and Y co-ordinate of each point, mean centre and standard deviation of ellipse was drawn for each PHC. The results showed that the disease is more clustered in the western part of the study site. Soil adjusted Vegetation Index (SAVI) was calculated to measure the vegetation. The value of SAVI varied from -0.42 to 0.69 (Mean – 0.13; SD – \pm 0.32). Land use/ land cover characteristics were extracted from 60 sites (e.g. 2x2 km²) of Kala-azar endemic villages. Spatial auto-correlation between the affected villages was measured for 5 PHCs of the study site (Table 1).

The study is in progress to estimate vector density through CDC light trap and man held aspirator technique at different environmental condition and study micro-climate and micro-habitat indicators for sandfly and geo-statistical analysis of the remaining PHCs.

Name of the PHC	Moran's Index	Expected Index	Varaiance	Z-score	Remarks
Motipur	0.005	-0.015	0.0003	1.31	Moderately clustered
Sahebganj	0.008	-0.021	0.006	1.18	While somewhat clustered, the pattern may be due to random chance
Paroo	-0.024	-0.026	0.0012	0.04	Random
Kurhani	0.003	-0.015	0.0002	1.27	While somewhat clustered, the pattern may be due to random chance
Saraiya	0.003	-0.016	0.0004	0.96	Slightly dispersed

EXTRAMURAL STUDIES

Epidemiology

1. Towards more cost effective Visceral leishmaniasis (VL) case detection and case management in endemic districts – Implementation strategies - PHASE-III

(Sponsor: WHO/ TDR)

P. Das et al.

Objectives:

- To test alternative strategies for active case finding for VL and PKDL and compare costs, yield of cases, feasibility and other factors across varying contexts. The strategies to be tested are:
 - Camp approach
 - Focal approach (neighborhood search around house of index case)
 - Blanket approach (house to house search)
 - Incentive based approach
- To analyze in real life settings the prospects and constraints of VL management, including drug availability, compliance, options for DOT (direct observed treatment), provider's treatment skills, clinical and biochemical monitoring and identification /management of adverse and serious adverse events, early referrals mechanisms, patient satisfaction and costs.
- To develop policy recommendations for improving early VL case detection and case management.

Progress:

The study was conducted in Parsa and Amnaur block of Saran district as intervention and control area respectively. The various case detection approaches investigated were camp, Index and Incentive approach as compared to the blanket approach as gold standard. In intervention block, a group of villages from 4 highly endemic sub-centres i.e. Maker, Jagdishpur, Pachrukhi and Bheldi were selected to cover near about 10 000 population in

each sub-centre area. The approaches applied in intervention area were Camp, Index and Blanket whereas in the control area it is incentive and blanket only.

Camp approach:

The camps' places were identified with a view to cover entire population. Some small adjoining villages were covered by the common camps and some large villages required camps at multiple place. Prior to camp, IEC were made through poster display, pamphlets distribution and audio canvassing. Altogether 37 days camps were organized in two rounds to cover 4 sub-centres consisting of about 40,000 populations. Altogether 651 individuals (48% male and 52% female) attended the camps for health check up in two rounds. A total 109 fever cases were screened through rK39, out of which 15 were found positive (14%) and referred to PHC. Six individuals having past history of VL and one having past history of PKDL were also identified. No suspected case of PKDL was indentified during camps.

Blanket approach (Household survey):

Immediately followed by the camp approach, household survey was conducted in the respective villages through the trained ASHA Health workers. Two round household's survey were conducted in control area and one round in the intervention area.

Table: Distribution of the vital characteristics

Characteristics	Parsa (Control)		Amnaur (Incentive area)
	Round 1	Round 2	Single Round
No. of HH	7927	7926	8914
Pop. Screened	47862	48414	42874
Male	25864	26109	23501
Female	21998	22305	19373
Age (Yrs.) Mean	24.7	24.4	24.4
Age (Yrs.) SD	18.5	18.5	17.9
No. of Known VL Cases	127	32	100
Male	72	17	53
Female	55	15	47
No. of Fever cases	15	18	30
rK39 +ve (new)	1 (6.7%)	3 (16.7%)	4 (13.3%)
No. of known PKDL cases	8	0	2
No. of suspected skin Lesion	4	2	12

Incentive approach:

This approach was also conducted only in the intervention area prior to blanket approach. Altogether 42,874 population was covered under this approach and 114 suspected VL patients referred to the PHC for diagnosis and treatment, out of which 21 cases (18%) were found rk39 positive and treated with miltefosine at PHC level.

Index case approach:

Passive case list (n=79) of the study area PHC during the specified period of one year and their respective households were marked as Index households after database search. The surrounding household within the radius of 50 meters of the index households were surveyed to trace focal cases against each index household. In two rounds of this approach, no new VL case was found.

Apart from these, 9 physicians (7 from Public and 2 from private set up) treating Kala-azar cases were interviewed. Mean years of work experience and VL experience was 16 and 9 yrs respectively. In last one year, the mean number of VL cases treated by them was 23.3. So far diagnostic tool is concerned, all stressed for rK39 followed by parasitological confirmation (33%). All advocated for Miltefosine, but the physician from Pvt. Sector also stressed for Amphotericin B. The main side effects of miltefosine, uttered by the treating physicians, included diarrhea, vomiting, renal toxicity and teratogenicity. The main problem regarding home management of VL came up was irregular treatment and difficult management of adverse events. One respondent responded that DOTs can be effective for home management. Doctor-patients interaction (10), patients satisfaction (29), health facility observation (4), DOT provider interview (15) and Miltefosine patients interviews (15) were also conducted.

The cost data analysis revealed that cost per camp come to 267 US\$ while cost per new VL case through camp was 1013 US\$. On the other hand cost of index case approach, incentive approach and cost per household were 800, 893 & 0.41 US\$ respectively while cost per new VL case through incentive and HH were 223 and 1269 US\$. The work is completed.

2. Enhanced VL case detection and improved case Management by the National Kala-azar Programme in Bangladesh, India and Nepal – Phase IV. (WHO/TDR)

P. Das, N Kumar et al.

Objectives:

- To enhance VL case detection and improved case management by the national kala-azar programme through implementation research in relation to newly established case detection/case management strategy

Specific objectives

- To analyze the feasibility, acceptability, cost, performance and results of a stratified case detection system (PCD and/or ACD) in the national kala-azar programme
- To analyze the prospects and constraints of health services based/home based VL case management through the national kala-azar programme including drug availability, option for DOTs (Direct Observed Therapy) by family members, providers' treatment skills, clinical and biochemical monitoring and identification/management of adverse events, patient satisfaction and cost.
- To develop policy recommendation for enhanced case detection and improved case management at national level.

Progress:

Study area has already been selected for camp as well as index case approach towards VL case detection. Data capturing tools has been finalized and got printed. First round training Programme to district kala-azar programme officer as well as MO, I/C and key personnel involved for the active case detection has been done. MO, I/C and other key personnel imparted the first round training to the personnel involved in active case detection at PHC/Sub-centres level in the selected areas through SOP for active case detection towards camp and index approach. Active case detection through index case approach is being initiated in the last week of November 2010 in the selected areas.

National Kala-azar Programme planned to conduct camp activity during the first/second week of December. Assessment of Camp approach will be conducted as soon as camp activity implemented by National Kala-azar Programme. Self administered KAP questionnaire for all health staff/ ANM/ASHA before start of training course and at the end of training course were conducted. In addition to above few physician interview, facility observation interview, patient's interviews and KAEP Manager Interviews were also done.

3. Estimating the annual incidence of kala-azar in two highly endemic blocks of Bihar: A pilot study comparing Snowball and house-to-house survey.

(Sponsor: World Bank)

N.A. Siddiqui et al.

General objectives:

- To obtain detailed baseline information of the programme implementation indicators in terms of
 1. Disease prevalence
 2. Early diagnosis and treatment and
 3. Vector control.

Specific objective:

Baseline survey

To estimate the proportion of

1. diagnosed kala-azar cases completing the standard treatment as per the national guidelines
2. suspected cases of kala-azar diagnosed by rK-39
3. houses in targeted kala-azar endemic areas covered with effective insecticide spray
4. blocks that do not have rK-39 test for kala-azar and/or first line medicine stock out during the last three months

End line survey

1. To evaluate whether at least 50% of the sample blocks achieved the goal of elimination programme i.e. 1 case per 10 000 population.

Progress:

The preparatory phase of the study has been initiated that includes selection of study area as per the study protocol, mapping of all the villages under the selected PHCs, development and printing of the data capturing tools. Training for the field workers and supervisors will be started shortly followed by actual survey.

4. Sentinel surveillance of Visceral leishmaniasis in endemic areas of Bihar.

(Sponsor: World Bank)

Alok Ranjan et al.

Objectives

The main objectives of setting up sentinel surveillance in the endemic regions of Kala-azar in Bihar are as follows:

- To develop a system for the generation and sharing of reliable and complete information on kala-azar from sentinel surveillance sites.
- To guide the program for evolving the most suitable strategy for effective sentinel surveillance for the kala-azar elimination involving the both public and private sectors

Impact objectives:

- To set up a Functional model or system of 18 sentinel sites in 4 selected pilot districts
- To enable these sites to generate information for appropriate action at different levels of health system.
- To validate the information emerging from the health management information system (HMIS).
- Based on sentinel surveillance system experiences, make recommendations for
 - a. further expansion of sentinel surveillance system (if required), and
 - b. Incorporation of key lessons learned into HMIS program.

Progress:

This project has been finally approved by the World Bank Experts. Initial funding has been received to start the project from January 2011. It has been planned to develop eighteen sites (18) in four high endemic districts namely – Muzaffarpur, East Champaran, Saran and Saharsa for the collection of data, monitoring of VL/PKDL subjects being treated at these sites and developing follow-up mechanism at these sites involving peripheral health workers and volunteers. Patient Information Card has been designed.

Clinical Studies

5. Treatment Response of Kala-azar/ HIV co-infected patients with Ambisome and Anti Retroviral Therapy. (Sponsor: MSF, Spain)

K. Pandey et al.

Objectives:

Primary Objective:

- To determine the efficacy and safety of Ambisome and ART in the treatment of Kala-azar/ HIV co-infected patients

Secondary Objective:

- To assess initial cure or clinical response for VL at 30 days after end of treatment

Progress:

A total of 55 patients (83.6% Males, and 16.4% females, median age group 35 yrs.) were included in the study. Of these 61% were migrant labourers. The Kala-azar/ HIV co-infected patients included in this study were treated with AmBisome in the dose of 5 mg/kg body weight for 4 days followed by anti-retroviral therapy. Median CD4 cell count at the time of kala-azar diagnosis was 66 cells/ μ l (Range - 38-112). Survival by one and two years after kala-azar treatment was estimated at 92%. None of the patients had initial treatment failure. Twenty seven (49.9%) had kala-azar relapse. A probability of KA relapse of 0%, 9.2% and 28.8% were found at 0.5, 1 and 2 years after KA treatment respectively. CD4 count less than 200 cells/ μ l at six month after ART initiation was predictive of subsequent relapse. The CD4 cell count at one and two years after ART initiation was 233 and 261 cells/ μ l respectively. No serious adverse events were observed during treatment.

6. Management of Visceral leishmaniasis cases co-infected with tuberculosis with AmBisome and anti-tuberculous drugs.

(Sponsor: MSF, Spain)

Nawin Kumar et al.

Objectives:

- To confirm the diagnosis of VL and tuberculosis in cases of MSF referred such suspected co-infected cases.
- To treat the confirmed VL/ TB co-infected cases with liposomal amphotericin B in the dose of 5 mg/Kg body weight for 4 consecutive days for VL and standard dose of Anti-tuberculous Drugs (ATT) for TB.
- To assess the initial cure and follow up for assessment of final cure.

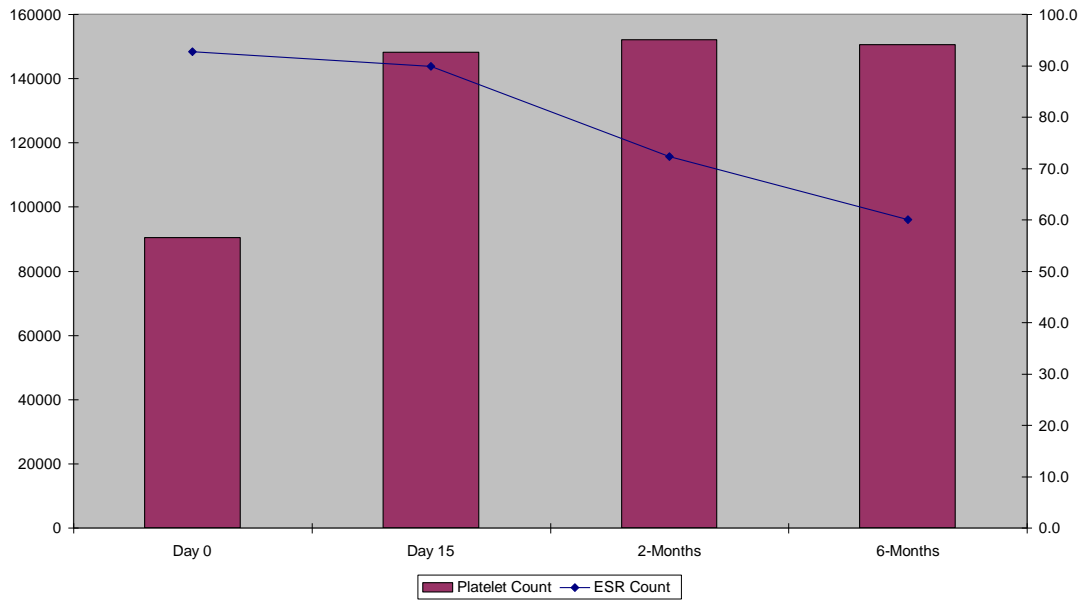
Progress:

A total of 23 confirmed cases (Male 16, Female 7) of Visceral leishmaniasis (VL) co infected with tuberculosis were enrolled in the study after assessment of inclusion and exclusion criteria. All patients were parasitologically confirmed for VL through splenic aspiration. Diagnosis of tuberculosis was confirmed by sputum examination in 8 patients and chest x-ray in 15 patients (pleural fluid aspiration in 7 patients).

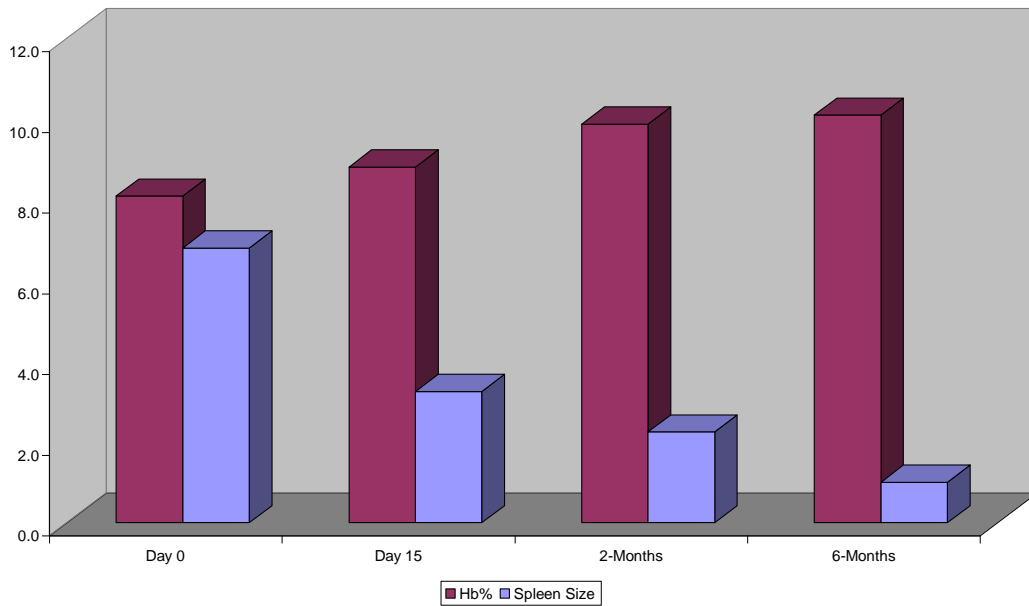
All the enrolled subjects were treated with Ambisome in the dose of 5 mg/kg body weight per day for 4 consecutive days, followed by ATT from day 5. All 23 patients completed full course of Ambisome and all were found initially cured for VL. Out of 23 patients, ATT treatment of 6-months duration, were completed in 12 patients and found initially cured for TB and finally cured of VL. Rest are under treatment for TB and simultaneously 6-months follow up for VL.

Assessment of spleen size regression and pathological parameters viz. Total WBC count, Hb%, Platelet and ESR showed suggestive improvement. All the hepatic and renal function tests were found within normal range during treatment and follow up period indicating lack of any adverse effect of Ambisome and ATT.

Avg. Platelet & ESR Count at different timepoints



Avg. Hb% & spleen size at different timepoints



The study is in progress.

7. A Prospective, Multicentric, Randomized, Two Arm, Open-label Phase III study to Assess Efficacy and Safety of Infusion of Amphomul[®] (Amphotericin B Emulsion) as Compared to AmBisome in Patients of Visceral Leishmaniasis (Kala-azar)

(BSV-AMBE III-KA-0908)

(Sponsor: Bharat Serums and Vaccines Ltd)

K. Pandey et al.

Objectives:

Primary objective

- To compare and evaluate efficacy of Amphotericin B emulsion (15 mg/kg/day as a single dose infusion) compared to AmBisome (15 mg/kg as a single dose infusion) in the treatment of Visceral Leishmaniasis (Kala-azar)

Secondary objectives

- To assess the safety of Amphotericin B emulsion (15 mg/kg as a single dose infusion) compared to AmBisome (15 mg/kg as a single dose infusion) in the treatment of Visceral Leishmaniasis (Kala-azar).

Progress:

Out of a total of 206 screened patients of suspected visceral leishmaniasis, 119 underwent randomization (79 male, 40 female). 90 patients were in the test group (Amphomul) and 29 in the control group (AmBisome). In the test group, 5 had initial treatment failure and 8 had relapse whereas in the control group no treatment failure or relapse occurred. All the 119 patients had undergone 30-days follow up whereas 108 have completed 6-months follow up. 114 patients had initial cure whereas 95 patients achieved final clinical cure up till now. No SAE has occurred till date whereas mild adverse events like rigor, fever etc occurred in a few patients.

8. Safety and efficacy of Liposomal Amphotericin B (Ambisome) in patients with Post Kala-azar Dermal leishmaniasis (PKDL)

(Sponsor: MSF, Spain)

P.K. Sinha et al.

Objectives:

Primary objective

- To assess liposomal amphotericin B regimens 2.5 mg/kg/bw for 20 consecutive days duration for their curative potential in PKDL (parameter: rate of patients with macular/nodular and papular lesions who achieve negative parasitological and clinical severity score of zero 12 months after end of treatment).

Secondary objectives

- To characterize the safety of liposomal amphotericin B when used for periods up to 20 days in three courses at the interval of 15 to 30 days.
- To assess the rates of initial response in relation to duration of treatment.
- To assess the rates of relapse after initial response
- To assess the clinical response of facial erythema and mucosal lesions

Progress:

Altogether 46 parasitologically confirmed PKDL cases, meeting all the inclusion and exclusion criteria as per the study protocol, were enrolled in the study. Out of 46 PKDL cases, 42 had past history of VL. The enrolled patients were treated with liposomal Amphotericin B (Ambisome) in the dose of 2.5 mg/kg body weight for 20 consecutive days in the repeated course. At the end of 1st course, 39 were found parasitologically negative but with the persistent skin lesions. Till date, 43 patients completed 2nd course and 25 had completed 3rd course of treatment. Out of 43 cases in 2nd course, 27 were found parasitologically negative but with less clinical severity. It was observed that even after parasitological cure, there were long lasting skin lesions though in the diminished form. The parasitologically negative patients with persistent skin lesions are under follow up to observe disappearance of lesions. No adverse side effect was observed, except in one case who developed Gullian-Barrie syndrome after 1st course of treatment. The study is in progress.

Table: Parasitological score at different time points

Parasitological Score	Base line	After 1st course	After 2nd course
0	4	39	39
1+	33	4	0
2+	9	0	0
3+	0	0	0
4+	0	0	0
5+	0	0	0
Total	46	43	39

Table: Clinical severity score at different time points

Clin. severity score	Base line	After 1st course	After 2nd course
0	0	0	3
1	0	15	12
2	1	12	0
3	4	14	0
4	4	2	0
5	2	0	0
6	35	0	0
Total	46	43	15

Basic aspect

9. Development of a DNA vaccine for Visceral leishmaniasis. (Leish DNA VAX)

(Sponsor: European Union)

P. Das et al.

Objectives:

- To identify sites for carrying out clinical trials.
- Preparation and training for monitoring the trials of Leish DNA Vax.
- To carry out the immunological studies on animals with IICB.
- To participate in immunological studies on human cells.

Progress:

A total of 160 PBMC, of which 80 treated, 20 fresh, 20 asymptomatic VL cases and 40 healthy controls, were sent to Berlin. Antigenic stimulation with 300 peptides were conducted and analyzed by ELISpot technique. Further work is under progress.

10. Laboratory based evaluation of rapid diagnostic tests for Visceral leishmaniasis.

(Sponsor: WHO/ TDR)

P. Das et al.

Objectives:

- To evaluate performance of VL diagnostics.
- To facilitate R & D of new VL diagnostics.
- To facilitate QA/QC in VL diagnosis in all endemic regions.

Progress:

This study was undertaken as Visceral Leishmaniasis Laboratory Network for evaluation of rapid diagnostic tests supported by the Diagnostics R&D unit of the UNICEF/UNDP/World Bank/WHO-TDR. Under this study, 6 diagnostic kits manufactured by 5 different manufactures (Table) were provided for RDT evaluation using archived

samples. Prior to selection of sera samples from our sera bank, DAT was standardized using 15 panel samples received from the Institute of Tropical Medicine Antwerpen, Belgium. After standardization of the DAT for proficiency testing, altogether 100 sera samples (50 DAT positive and 50 DAT negative) of our sera bank were selected and randomized for evaluation of different RDTs.

All the randomized samples were tested by all the six RDTs in two lots by two different laboratory technicians following the same SOP. In Lot 2, only 25% of the randomized samples were tested. The test results and other details were entered into the computer using Epi-Info version 3.1. Data analysis is under process by the sponsor.

Table: Details of RDT Kits and Testing results of Lot 1 AND Lot 2

Manufacturer	Product	Lot 1				Lot 2			
		Technician	-Ve	+Ve	Total	Technician	-Ve	+Ve	Total
Span	CRYSKA	1	58	42	100	1	16	09	25
		2	57	43	100	2	14	11	25
	SIGKA	1	49	51	100	1	11	14	25
		2	49	51	100	2	11	14	25
Chembio	DPPLEISH	1	50	50	100	1	10	15	25
		2	50	50	100	2	09	16	25
Inbios	KALADET	1	50	50	100	1	13	12	25
		2	50	50	100	2	12	13	25
Diamed	DIAMEDIT	1	49	51	100	1	10	15	25
		2	49	51	100	2	11	14	25
CTK Biotech	ONSITE	1	50	50	100	1	13	12	25
		2	50	50	100	2	13	12	25
Total			613	587	1200		143	157	300

Vector Biology & Control

11. Usefulness, Feasibility and Cost of Vector Control Monitoring in Kala-azar Endemic District of Bihar, India – Phase III Study.

(Sponsor: WHO/TDR)

V. Kumar et al.

Objectives:

- To assess the usefulness, feasibility and costs of the Monitoring Toolkit developed in Phase II for quality control of IRS, including real coverage, spraying performance.
- To evaluate some specific indicators in the toolkit that require further evidence before large –scale application, its output and impact of IRS.
 - To establish the **norm** for efficient use/application of insecticides (in terms of gm per household and eventually per population) for IRS.
 - To select the most appropriate tool (or tool mix) amongst three different IRS **entomological** monitoring indicators: i. the filter paper method (chemical residue on filter papers as tracers in houses), ii. The bio-assay method and iii. Sandfly captures by CDC light traps for reduction of sand fly density.
 - To evaluate the **outcome of IRS** through assessing the insecticide residual action of IRS based on repeated sand fly collections after IRS compared to control.
- To compare the user friendliness and efficiency of two pumps (Hand compression and Stirrup) in terms of number of houses sprayed per day, volume of insecticides used per pump and the operational feasibility of each pump.

Progress:

In continuation of the study with an objective to compare the user friendliness and efficiency of two pumps (Hand compression and Stirrup) and the operational feasibility of each pump, a site specific study was carried out in Gorigawan village of Mahua PHC, Dist. Vaishali where targeted population for IRS was 3331 spread over 663 targeted households. Before start of the spraying operation, the spray men were given one-day training on difference of the two pumps as well as spraying operation. There were two spray teams in action and both the teams were provided with PPE like apron, gloves, goggles, mask and caps. Specified filter papers were also made available for assessment of DDT concentration. After 5-days scheduled IRS activity, 3249 population (97.5%) in 605 households (91.3%) were covered.

Sandfly density

Pre-spray sandfly collection was made 2 weeks prior to IRS and post-spray density was carried out after 2 weeks, 3 months and 5 months of IRS in the intervention and sentinel

households. Prior to spray 17 sandflies were collected from both intervention and sentinel households whereas during post-spray collection at different time points 4, 13, 14 and 20, 18, 21 sand flies were collected respectively.

Residual activity (Bio-assay test)

Bioassays were done independently by insect collectors/ entomologist for measuring the efficacy of IRS (DDT) after 3 and 5 months of spraying. Bio-Assay result after 3 and 5 months of IRS in intervention HHs of experimental village was found 36% and 25% respectively.

Filter Paper chemical analysis for DDT concentration

Filter Papers as per WHO recommendation were pasted along with mimic papers of different colours without the knowledge of spray men at different height on the four walls of the room. After DDT spraying the filter papers were collected. A total of 5 dried and packed filter papers were preserved for chemical analysis through three different laboratories abroad named Liver pool, Vimta lab and Belgium. But sample analysis revealed inconsistency of DDT concentration.

Table: DDT chemical analysis by Liver pool, Vimta Lab. and Belgium laboratory

Sample No.	Liver pool (gm/m²)	Sample No.	Vimta lab. (gm/m²)	Sample No.	Belgium (gm/m²)
1A	0.39	1B	0.01987	1C	1.09
2A	0.71	2B	0.03004	2C	1.88
3A	0.33	3B	0.00290	3C	0.87
4A	1.41	4B	0.00281	4C	0.67
5A	0.53	5B	0.00303	5C	1.66

Comparison of Stirrup and Compression Pump

Based on the logistics and operational observations, it was found that hand compression pump is better than stirrup pump as the first one cost lesser and larger coverage as compared to stirup up. Stirring is not required in hand compression pump and there is very negligible wastage of insecticide.

Table: Stirrup vs hand compression pump

S.No.	Statements	Stirrup pump	Hand compression pump
1.	Cost	92 USD (1 \$ = 46)	81 USD (1 \$ = 46)
2.	Per day can cover one squad (HH)	45 – 77	56 - 79
3.	Pump used in one squad	2	3
4.	No. of Stroke	23 – 27/min	20 in initial stage
5.	Nozzle discharge rate	650 – 750 ml/min	750 ml/min
6.	Stirring	Required while spraying	Not required
7.	No. of stroke required to empty of each full tank	Unlimited	70 – 85
8.	Time required to empty one tank (in min)	30 – 40	20 – 25
9.	Wastage of Insecticide suspension (DDT)	100 – 200 ml/pump	Very negligible
10.	HH covered in 5 days per squad	280	325
11.	Population covered in one day	238-345	322-432
12.	Population covered in 5 days	1464	1795
13.	Square meter covered by per pump per day	293	214

12. Evaluation of the feasibility and usefulness of a monitoring and evaluation toolkit for VL vector control in national programmes (Phase IV). (WHO/TDR)

V. Kumar et al.

Objectives:

General objective:

- To contribute to an effective IRS (Indoor Residual Spraying) Programme in the context of the VL elimination initiative through the evaluation of a newly developed M&E toolkit which guides programme managers to respond quickly to different programmatic shortfalls and can be integrated into a wider web based “VL Decision Support System”.

Specific objectives:

- To evaluate the applicability and user-friendliness of the M & E toolkit in the national IRS program for VL vector control

- To analyze the response of the vector control program to issues identified by using the M &E toolkit
- To use the information generated by the application of the standardized M &E toolkit for a comparative analysis of the IRS programs in the 3 countries

Progress:

First training programme (21st – 22nd and 27th – 28th Jan 2010)

First training on “Monitoring and evaluation Toolkit and Case detection” in two batches were organized wherein 50 District Malaria Officers (DMO) and Malaria Inspectors (MI) of 25 kala-azar endemic districts of Bihar attended.

The participants raised issues like lack of infrastructure, insufficient manpower, entomologist, technical knowhow of CDC light trap and Bio-Assay and sensitivity test. They agreed that monitoring of IRS is the weakest point and suggested for Hindi version of the toolkit for implementation.

Vector Study

Four districts were selected for vector study where IRS had been scheduled from 5th March 2010. The pre-spray activities involved estimation of Sand fly density in Intervention, Sentinel and Control Villages by CDC Light Trap and distribution of toolkit (Hindi version) in respective PHCs/Districts.

During the spray, spot visits to the PHCs were made to verify DDT availability, spraying operation, checking of pumps, nozzles, swath formation. Filter papers were distributed and collected after use. Officials at districts headquarter and PHCs were consulted for implementation of M&E toolkit.

After spray, sand fly density in Intervention HHs, Sentinel HHs and Control HHs of selected villages were re-assessed by CDC Light Trap method. Apart from this, other activity involved were community acceptance to IRS, visual observation of sprayed wall, constraints of toolkit and Bio-Assay test in Intervention HHs.

First follow up meeting (24th – 25th May 2010)

After first round workshop, first follow-up meeting was conducted after IRS wherein total 43 participants including DMOs, ACOMO, MIs, KTS of 18 endemic districts, Director in chief cum SPO, Bihar, ZMO Kala-azar also participated.

Some suggestions for further development in IRS Programme and Toolkit came out after discussion that includes fixed yearly schedule for IRS, uncomplicated and in-time funding procedure, pre-spray training at PHC level, enhanced logistic support including PPE, provision of vehicle at PHC level for monitoring, camp system for DDT storage and stay of spray men, wage revision for spray men, more stress on IEC with fund availability at the PHC level and delinking of personnel involved from other activities.

Second follow up meeting (10th – 13th Aug 2010)

In the second follow-up meeting there were altogether 38 participants from 20 districts of Bihar including DMOs, ACMOs, MIs, Director in chief cum SPO, Bihar, AD Kala-azar. Besides them, Dr. S.P. Singh from BHU and Mr. Rajib Chawdhury from WHO-SEARO also took part in focus group discussion (FGD).

Only two districts submitted the filled up M&E Toolkit, in rest of the districts, DMOs/MIs could not find time to implement the toolkit. But all of them opined for its utility if it is provided in local language. After FGD, it was observed that funding was the main problem for vector control operation followed by transportation of DDT and lack of manpower.

Some suggestions came out after FGD were approval of micro action plan 2-3 months prior to IRS, release of fund 15 days prior to IRS, adherence to pre-scheduled IRS duration, IRS supervision and monitoring at PHC level with more man power, incentive-based involvement of ASHA, camp facility at 4-5 km for easy operation of IRS activity, proper logistic support with 20% buffer stock, replacement of defunct articles like pumps, nozzles, buckets etc., pick-up van facility for DDT and spray men etc.

13. 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.

(Sponsor: ICMR Task Force)

S. Kesari et al.

Objectives:

- 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:

Overall, the proportion of *P. argentipes* in both endemic and non-endemic areas was significantly much higher ($P < 0.001$) as compared with the proportion of the *Sergentomyia* and *P. papatasi*. This indicates that the distinction of different species of sandfly is similar in endemic and non-endemic area.

The results of multilevel logistic regression analysis of demographic characteristics of endemic and non-endemic site showed that mud plastered wall (P value = 0.004), thatched roof (P value = 0.008), mixed dwelling (P value = 0.015) and area (P value = 0.113) were found associated with presence of absence of vectors. The final model indicated that the type of house wall, especially mud plastered wall (OR-1.71; P -value = 0.001), mixed dwellings i.e. cattle and human being (OR-1.60; P -value = 0.002) living together in high endemic area (OR-2.22; P -value = 0.001) of Kala-azar could be the possible independent risk factors for presence of vector population without having any significant joint effect.

IRS 1D LISS-IV scenes for both the site were obtained, rectified and mosaiced. A Landuse/land covers characteristic of endemic region (Vaishali district) was generated for the endemic region. The outcome of factor analysis confirm that breeding preferences of *P. argentipes* for landscape element, including minimum NDVI, swampy land and orchard/settlement produced high loading while analyzing with density of *P. argentipes* in an endemic region, whereas, waterbody and dense forest in non-endemic site.

Soil properties between two districts were studied, indicating higher pH and high soil moisture content in the study site. Finally, software has been developed at RRSSC Kharagpur collaboration with RMRIMS for determining the Kala-azar ‘endemic’ and ‘non-endemic’ region.

The study is being continued to extract the seasonal land use /land cover characteristics of endemic and non-endemic region, derived through IRS-1D LISS IV and to validate the previous model to improve the accuracy level.

D. Others

I. Supportive Activity

Epidemiological profiling of HIV/AIDS situation at district and sub-district level using Data Traingulation (Phase-II) – RMRI as Nodal Centre (A NACO approved and PHFI, India sponsored project)

Introduction:

National AIDS Control Organisation (NACO) has recently undertaken a project titled 'Epidemiological Profiling of HIV/AIDS Situation at District and Sub-district Level Using Data Triangulation' in seven states (182 districts), with the objective of developing district HIV/AIDS epidemic profiles and finding answers to some important programme questions. Rich information is currently available under NACP-III from multiple sources such as HIV Sentinel Surveillance, programme data from ICTC, PPTCT, STI, ART, BB & TI, mapping of HRG, etc.

This project aims at synthesizing this large evidence base to give inputs for effective planning and implementation of programme at district level. This project also aims at building the capacity of the state and district program managers and M & E persons in data analyses, triangulation and use for program review and planning.

This will also contribute to refining district prioritisation as well as revising the Annual Action Plans of NACO & State AIDS Control Societies (SACS). RMRIMS, ICMR has been identified as STATE COORDINATING AGENCY (SCA) for the implementation of this project in Bihar State.

Progress:

A three-day National Workshop was organized from 21st January to 24th January 2010 at Lucknow for imparting training on data triangulation to persons working on behalf of SCA. For the capacity building, First state level workshop was organized for the officials working at 22 districts out of 38 district in Bihar during 28th June to 3rd July 2010 at RMRI in collaboration with NACO, BSACS and PHFI. The districts, where HSS sites were not available or having very few HIV testing sites, did not participate in the workshop. Though the data of these districts were made available to SCA through BSACS.

All other data were received from BSACS through CMIS. Data related to HSS, Blood Bank, ICTC, PPTCT, ART, STD, Mapping of HRG, etc of all 38 districts were extracted from CMIS-database of NACO, and separate databases for each 38 districts were compiled for validation and rectification. After the workshop, the participants were asked to collect data from all sources for their respective districts for data validation and completeness. The participants in each district conducted field work for collection and compilation of data mainly for ICTC and PPTCT.

A second state level workshop was organized for those district officials who participated in first state level workshop during 9-12 th August 2010. The participants were asked to check for the accuracy, completeness and validation of the data of their districts, compiled by RMRI and collected from the field by the officials. After complete screening of each data set, a final tabulation for each district was compiled.

HSS and cleaned ART data were analyzed using Stata version 10. The analyzed data were used for final compilation of HSS and ART data for each 38 districts. Cleaned and Final Tabulation of 38 districts has been sent to NACO, New Delhi for verification.

Final analysis is in progress.

II. Meetings/ Trainings/ Workshop/ Symposium held at the Institute

- Workshop on “Advanced separation technology” organized by NIPER, Hazipur and RMRI from 27th – 31st Jan 2010.
- Training programme on Vector Monitoring and Evaluation (M&E) and VL Case detection held from 21st – 22nd and 27th – 28th Jan. 2010.
- A scientific talk on “Developing New Tools to combat insecticide resistance” delivered by Dr. Mark J.I. Paine, Senior Lecturer, Liverpool School of Tropical Medicine on 09th Feb 2010.
- A scientific lecture on “Epidemiology of HIV/AIDS” delivered by Prof. Roger Detels, Dept. of Epidemiology, UCLA School of Public Health on 11th Mar 2010.
- DST meeting for Amphotul Phase III study, sponsored by Bharat Serum & Vaccine Ltd., held on 20th Mar 2010.
- Workshop on Flow cytometry, organized by Dept. of Immunology, RMRIMS, held on 27th Apr – 01st May 2010.
- WHO/TDR sponsored First Follow-up meeting with DMOs/MIs of Kala-azar endemic districts on “Problems identified during IRS and Case detection”, held from 24th – 25th May 2010.
- Workshop on “Application of Bio-informatics in Medical Sciences”, organized from 29th – 30th June 2010.
- First State Level Workshop on “Epidemiological Profiling of HIV/AIDS Situation at District and Sub-District Levels using Data Triangulation (Phase – II)”, sponsored by NACO and PHFI held from 28th June – 3rd July 2010.
- Workshop on “BD Discovery Labware”, sponsored by BD Biosciences India, held on 09th July 2010.
- First Convocation of NIPER, Hajipur, held on 7th August 2010 at L.N.M. Institute of Economic Development & Social Research, Patna.
- Second State Level Workshop on “Epidemiological Profiling of HIV/AIDS Situation at District and Sub-District Levels using Data Triangulation (Phase – II)”, sponsored by NACO and PHFI held from 9th – 12th August 2010.
- RMRI and WHO/TDR sponsored Second Follow-up meeting cum Training Workshop Programme on “Quality Assured Vector Management and Case

Detection in relation to Kala-azar Elimination programme in Bihar, India”, held from 10th – 13th Aug 2010.

- World Bank/ NVBDCP sponsored Training to Kala-azar technical Supervisors (KTS) – 1st Batch held from 16th – 26th Aug 2010.
- A scientific talk on “Genetics of the mechanism of antibiotic resistance and integrons – the Gene Capture System” delivered by Dr. Amit Ghosh, Emeritus scientist, NICED, Kolkata on 20th Aug 2010.
- Meeting with officials of World Bank and NVBDCP for progress review of World bank sponsored projects was held on 14th Sept 2010.
- World Bank/ NVBDCP sponsored Training to Kala-azar technical Supervisors (KTS) – 2nd Batch held from 17th – 26th Sept 2010.
- World Bank/ NVBDCP sponsored Training to Kala-azar technical Supervisors (KTS) – 3rd Batch held from 20th – 29th Oct 2010.
- Workshop on “Application of Bioinformatics in Medical Sciences with an introduction to SOLiD”, held from 22nd – 23rd Oct 2010.
- A scientific talk on “The role of alfa-tocopherol succinate and other molecules to protect from radiation” delivered by Dr. Vijay Kumar Singh, Scientist, Armed Forces Radiobiology Research Institute, University of the Health Sciences, Bethesda, USA on 11th Nov 2010.
- World Bank/ NVBDCP sponsored Training to Kala-azar technical Supervisors (KTS) – 4th Batch held from 19th – 28th Nov 2010.
- A scientific talk on “Improving T cell responses during experimental visceral leishmaniasis” delivered by Dr. Christian Engwerd , Dept. of Immunology & Infection Lab., Queensland Institute of Medical Research, Brisbane, Australia on 25th Nov 2010.
- Dr. Farokh Modabbar, DNDi, Geneva, Switzerland was honoured with Deshratna Dr. Rajendra Prasad Memorial Oration Award 2010 on 6th Dec 2010 for his scientific talk on “Leishmaniasis Vaccine – Past, Present and Future”.

III. Meetings/ Trainings/ Workshop/ Symposium attended

- Ganesh Sahoo, Scientist “C” attended “The Eighth Asian Pacific Bioinformatics Conference”, held at Banagalore from 18th – 21st Jan 2010.
- Dr. VNR Das, Scientist ‘D’ attended “National workshop on epidemiological profiling of HIV/AIDS situation at District and sub-district level using data triangulation”, sponsored by NACO, APAC, VHS and USAID at Dept. of Hospital Administration, CSM Medical University, Lucknow from 22nd – 24th Jan 2010.
- Mr. Alok Ranjan, Scientist ‘C’ attended “National workshop on epidemiological profiling of HIV/AIDS situation at District and sub-district level using data triangulation”, sponsored by NACO, APAC, VHS and USAID at Dept. of Hospital Administration, CSM Medical University, Lucknow from 22nd – 24th Jan 2010.
- Mr. R.B.Verma, T.O. attended “National workshop on epidemiological profiling of HIV/AIDS situation at District and sub-district level using data triangulation”, sponsored by NACO, APAC, VHS and USAID at Dept. of Hospital Administration, CSM Medical University, Lucknow from 22nd – 24th Jan 2010.
- Dr. K. Pandey, Scientist ‘D’ attended the Workshop on Pharmacovigilance, held at Hotel Diamond, Varanasi on 16th Feb 2010.
- Dr. K. Pandey, Scientist ‘D’ participated in the Training on “Alternative First Line ART, Second Line ART for Paediatrics, EID and TB & HIV”, held at Dept. of Medicine, Institute of Medical Sciences, BHU, Varanasi on 12th – 13th Mar 2010.
- Dr. P. Das, Director attended ICMR – DNDi meet held at ICMR Hqd on 19th Apr 2010 and presented the Institute’s experience of anti-leishmanial drugs for treatment of VL.
- Dr. P. K. Sinha, Scientist – E attended ICMR – DNDi meet held at ICMR Hqd on 19th Apr 2010 and presented the “Key points prior to implementation of treatment option”.
- Dr. VNR Das, Scientist D attended the Joint meeting on Swine flue and Dengue, held at Bihar State Health Society, Patna on 15th Sept. 2010.
- Dr. Krishna Pandey, Scientist D attended the Joint meeting on Swine flue and Dengue, held at Bihar State Health Society, Patna on 15th Sept. 2010.

- Mr. N. Kumar, Scientist – E attended Pre-conference Training Course on “Health System Research”, held at NIHFW, New Delhi on 10th Nov. 2010.
- Dr. N.A. Siddiqui, Scientist – B attended Pre-conference Training Course on “Health System Research”, held at NIHFW, New Delhi on 10th Nov. 2010.
- Mr. N. Kumar, Scientist – E attended 28th Annual conference of Indian Society for Medical Statistics, held at NIHFW, New Delhi and delivered a talk on “How do Health Care Providers deal with Kala-azar in Indian subcontinents”.
- Dr. N.A. Siddiqui, Scientist – B attended 28th Annual conference of Indian Society for Medical Statistics, held at NIHFW, New Delhi and delivered a talk on “Seasonal and spatial distribution of Kal-azar cases in Bihar, India”.

IV. Distinguished visitors

- Dr. Mark J.I. Paine 09 Feb 2010
Senior Lecturer
Liverpool School of Tropical Medicine, UK

- Dr. Krishna Kumari 09 Feb 2010
IICT, Hyderabad

- Prof. Roger Detels 11 Mar 2010
Dept. of Epidemiology,
UCLA School of Public Health, USA

- Sri Nand Kishore Yadav 28 Apr 2010
Honorable Minister,
Health & Family Welfare, Govt. of Bihar

- Dr. C.P. Thakur 28 Apr 2010
Rajya Sabha Member

- Dr. V.M. Katoch 28 Apr 2010
Secretary, Dept. of Health Research &
Director-General, ICMR, New Delhi

- Dr. N. Selva 15 – 16 Sept. 2010
Tuberculosis Research Centre (ICMR)
Chennai

- Dr. D.S. Chauhan 15 – 16 Sept. 2010
JALMA (ICMR)
Agra

- Dr. Vijay Kumar Singh 11 Nov 2010
Scientist
Armed Forces Radiobiology Research Institute
University of the Health Sciences
Bethesda, USA

- Dr. Christian Engwerd 25th Nov 2010
Dept. of Immunology & Infection Lab.
Queensland Institute of Medical Research
Brisbane, Australia

- Dr. Farokh Modabbar 6th Dec 2010
DNDi, Geneva
Switzerland

V. Publication

2010

1. Banerjee R, Kumar S, Sen A, Mookherjee A, Roy S, Pal S and Das P. TGF- β regulated tyrosine phosphatases induce lymphocyte apoptosis in *Leishmania donovani*- infected hamsters. *Immunology and cell Biology*, 2010.
2. Bhunia GS, Kesari S, Jeyaram A, Kumar V, Das P. Influence of topography on the endemicity of Kala-azar: a study based on remote sensing and geographical information system. *Geospat Health*. 2010; 4(2): 155-165
3. Bhunia GS, Kumar V, Kumar AJ, Das P and Kesari S. The use of remote sensing in the identification of the eco–environmental factors associated with the risk of human visceral leishmaniasis (kala-azar) on the Gangetic plain, in north–eastern India. *Annals of Tropical Medicine & Parasitology*, 2010; 104(1): 35–53.
4. Clements MF, Gidwani K, Kumar R, Hostomska J, Dinesh DS, Kumar V, Das P, Müller I, Hamilton G, Volfova V, Boelaert M, Das M, Rijal S, Picado A, Volf P, Sundar S, Davies CR and Rogers ME. Measurement of recent exposure to *Phlebotomus argentipes*, the vector of Indian visceral Leishmaniasis, by using human antibody responses to sand fly saliva. *Am J Trop Med Hyg*. 2010; 82(5): 801-807.
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3. Dr. K. Pandey, M.D.	Scientist D
4. Dr. Nawin Kumar, M.D.	Scientist C
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6. Smt. Marry Shanti	Staff Nurse
7. Smt. Raina Sinha	Staff Nurse
8. Smt. Ajita Kujur	Staff Nurse
9. Smt. Kalpana Kumari	Staff Nurse
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11. Mr. Umesh Kumar	Laboratory Technician

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3. Dr. D. S. Dinesh, Ph.D.	Scientist B
4. Mr. A. Jeyakumar	Research Assistant
5. Mr. N. K. Sinha	Technical Assistant
6. Mr. A. K. Mandal	Insect Collector
7. Mr. S. A. Khan	Field Assistant

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2. Mr. M. P.Thakur Technical Assistant
3. Mr. M. Prasad Technical Assistant
4. Mr. K. Chowdhary Animal Attendant
5. Smt. Geeta Devi Animal Attendant
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2. Mr. Udai Kumar, M.Com	A.O. (F&A)
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4. Mrs. Anita Kumari	Section Officer
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6. Mr. M.M.Ansari	Personal Assistant
7. Mr. S.N.Rabidas	Stenographer
8. Mrs. S.Kumari	Stenographer
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15. Mr. Manoj Kumar	LDC
16. Mr. Alok Kumar	Hindi Typist
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4. Mr. Jawahar Prasad	Plumber
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3. Mr. Ranjeet Kumar	Watchman
4. Mr. B. Murmu	Watchman
5. Mr. N.K. Chowdhary	Watchman
6. Mr. U.S. Singh	Watchman
7. Mr. Uday Shankar	Watchman
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