Kala Azar

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Abstract : Although visceral leishmaniasis (VL) or Kala-azar caused by *Leishmania donovani* (LD) is globally, a public health problem and its eradication is of high priority, yet until now, no animal reservoir has been found in India and transmission of Kala-azar occurs from man to man through the recognised vector *P. argentipes*. The cutaneous or dermal leishmaniasis is caused by *L. tropica* and this is restricted to Rajasthan where it is zoonotic. Investigation by Sharma *et al* revealed the presence of zoonotic reservoir in Indian desert gerbils, the rodent burrows of which yielded two species of sandfly. **(Indian J Pediatr 1999; 66 : 539-546)**

Key words: *Kala azar; Cutaneous; Zoonotic.*

In India, infection with *L. donovani* leads to several types of clinical as well as sub-clinical conditions. It is interesting to note that sub-clinical infection may be inapparent with transient fever and hepatosplenomegaly. This condition may further progress and develop as Kala-azar or it may spontaneously cure itself. On rare occasions, leishmaniasis may present itself as acute Kala-azar which may be confused with enteric fever by the beside. An instance of acute Kala-azar in 1950 in a school going boy, aged 16 years may be cited here. The boy, in his adolescent years presented first with high fever and a palpable spleen. Treatment by a general practitioner on the line of enteric fever did not produce any result. To add to the confusion, by the end of the first week the boy started bleeding profusely from the intestines. The presence of a clean moist tongue, absence of toxaemia and a fairly good appetite helped the author to arrive at a clinical diagnosis of acute Kala-azar. A microscopic examination of the buffy coat of the peripheral blood smear revealed the presence of amastigotes of *L. donovani*. A single course of treatment by intra-muscular injections of pentavalent antimonials for 3 weeks cured this type of acute Kala-azar and a follow-up study also revealed no recurrence.

Another case in a child with fever of 18 months long duration with a protuberant abdomen and marked emaciation was observed. The child was earlier treated as a case of Indian childhood cirrhosis but an in-depth clinical examination revealed that the spleen was adherent to the left lobe of the liver which could be separated by the palpating fingers and as such, Napier's aldehyde test was performed which came out to be strongly positive. On the basis of the clinical findings and the laboratory report, a diagnosis of Indian Kala-azar was made. The child recovered with only one course of first line drug, namely sodium antimony gluconate, which was given for a period of three weeks in gradually increasing doses.

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A word of caution : The aldehyde test is not a specific test for Kala-azar as it may be positive in Indian childhood cirrhosis and only a *very strong* aldehyde test will be helpful in differentiating the two conditions.

Another form of Kala-azar known as lymphatic leishmaniasis is characterised by fever and generalised lymph node enlargement without hepatosplenomegaly. This kind of presentation is mostly confused with glandular tuberculosis. It should be realised that visceral leishmaniasis (VL) is the severest form of the disease, and if left untreated, it may prove fatal. Post Kala-azar dermal leishmaniasis (PKDL) is the sequelae to the visceral development of the parasite which involves skin and mucus membranes. It usually appears 6 months to 18 months after apparent cure from Kala-azar. PKDL appears in three well recognised clinical forms, the earliest being hypopigmented lesions, then an intermediate erythematous type, and finally the well developed nodular type. The nodules may some times ulcerate leading to non-suppurative and non-tender ulcers. LD bodies can be easily demonstrated if a skin snip smear is made by scraping the material from the coalescing hypopigmented patches. This form of Kala-azar gets cured only after the patient receives three to four courses of antimony by the intra-muscular route, each course lasting for 3 to 4 weeks depending upon the severity of the case.

In yet another case, a 10 year old boy suffering from Kala-azar for two and half years in north Bihar was examined after he had failed to respond to well known anti-leishmanial treatment regimens. Even the drastic step of well planned splenectomy in Calcutta School of Tropical Medicine failed to cure him. He presented with signs and symptoms of meningitis following which a diagnostic lumbar puncture was performed on two consecutive days and both the samples revealed amastigotes of leishmania in the cerebrospinal fluid. The patient was cured with a prolonged course of amphotericin B given by the i.v. route as this drug is known to cross the blood-brain barrier. A seven year follow-up has revealed no recurrence.

A scientific article "Leishmanial Meningitis" was published on the basis of the information given above in the American Journal of Tropical Medicine in 1996 and also in the Lancet as a news item in the same year[2].

Another unusual presentation of leishmaniasis in children in India is the renal involvement[3]. Fifty one patients in a series of 1000 parasitologically confirmed cases of Kala-azar showing proteinuria were studied from the point of view of renal involvement. Forty two of these patients showed blood urea above 40 mg/dl and 12 had decreased glomerular filtration rate as well. Serum creatinine level was within normal limits in all patients showing more than blood urea of 100 mg/dl. It is therefore suggested that although there may be glomerular involvement in some cases of Kala-azar the changes are reversible with conventional treatment.

Another case of cerebral involvement was brought to light when a 20 years old male was referred to the Rajendra Memorial Institute of Medical Sciences by the Department of Medicine of Patna Medical College Hospital, where he was admitted for the treatment of generalised seizures[5]. He was thoroughly investigated as he showed hypopigmented patches all over the body following an attack of visceral leishmaniasis some 4 to 5 years back in

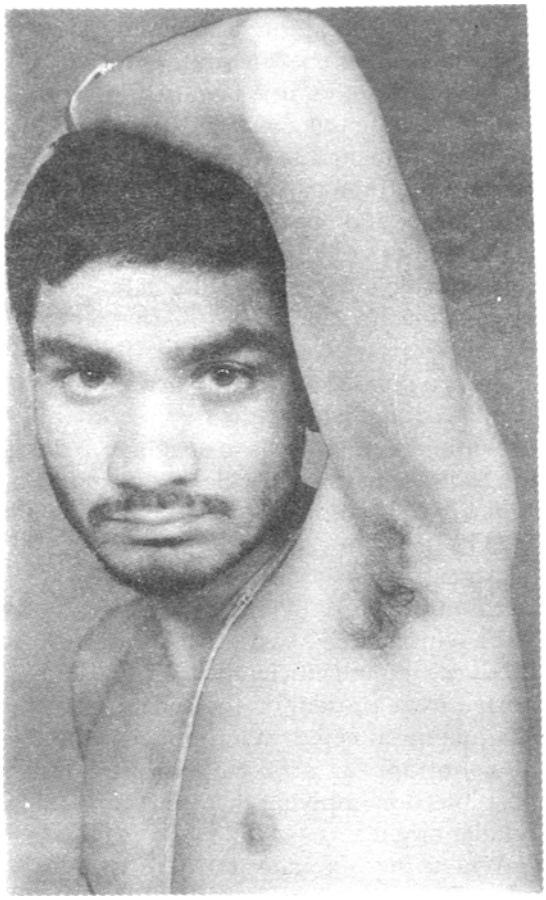

Fig. 1. A case of PKDL in a young man showing mini-nodular form of eruption on nose

Patna where he lived on the bank of the river Ganga. A detailed history followed by clinical examination revealed that the generalised seizures from which he suffered recently were actually caused by a process of cerebritis. On lumbar puncture, the CSF came out under pressure but the microscopic and cultural examination did not reveal L.D. bodies. However, when computerised tomography (CT) was done, there was definite evidence of cerebritis. The interesting observation is that the peripheral blood examination by the Polymerase Chain Reaction (PCR) was found to be positive for cerebral leishmaniasis. This young man was treated and cured by oral administration of ketoconazole, an antifungal drug known to cross the blood brain barrier. This oral medication was preferred as its concentration is reported to be greater in the central nervous system than amphotericin which we used when we treated our case of leishmanial meningitis. The patient is still under observation after being cured. The two instances of cerebral and meningeal involvement recorded here are unique examples of insult to nervous system which were not documented in the past by any research worker.

Pathological Features and Immunological Studies in Leishmaniasis

Indian Kala-azar is caused by *Leishmania donovani* and its multiplication by simple fission occurs in the reticuloendothelial cells of the liver and spleen which are greatly enlarged. Parasites known as L.D. bodies can be easily demonstrated either by tapping the bone marrow or by puncturing the spleen or occasionally by inserting a fine needle into the lymph nodes in the epitrochlear, cervical, the axillary or inguino-femoral group of glands (the author succeeded by inserting a fine needle into the small epitrochlear gland in the reported case of leishmanial meningitis). The aspirate from the lymph nodes is studied under the microscope and in culture for the presence of amastigotes and promastigotes respectively. A marked progressive granulocytopaenia and slowly progressive anaemia are the usual features of Kala-azar oc-

curring in epidemics in the different states of north-eastern part of India. There is a great increase of gamma globulin which is mainly IgG. Successful chemotherapy reverses these changes and only rarely does cirrhosis of the liver occur.

Immunological studies[1] : Clinical and epidemiological facts suggest that the human beings themselves are responsible for harbouring the leishmanial parasites and the reservoir of infection is built up from the patients of cutaneous leishmaniasis including PKDL and also from healthy carriers who are residing in the same family where some other members are suffering from Kala-azar. David Sacks in collaboration with Indian scientists at Rajendra Memorial Research Institute showed that these so-called healthy carries were able to respond to *L. donovani* soluble antigen by lymphocyte transformation in vitro or by skin testing (leishmanin test). Also that T-cell response tests were far better epidemiologic tools than ELISA test for the detection of asymptomatic infection. It is well known that *L. donovani* being an intracellular parasite defies direct killing by antibodies and a protective cell-mediated immune response is kept in abeyance until cure takes place. In all probability both the cell-mediated immune response and the antibody immune response operate at different levels with varying intensities to determine the clinical outcome.

LIPOPHOSPHOGLYCAN (LPG) IN VISCERAL LEISHMANIASIS

The major cell surface glycoconjugate of leishmanial parasites is lipophosphoglycan which is relatively abundant and has a unique structure. It is a heterogeneous glycoconjugate containing a repeating phosphorylated disaccharide unit, 16 of which are linked together in a linear array by alpha-glycosidic linkages. The disaccharide units are attached to a unique CHO core containing three galactose, two mannose, one glucose and one glucosamine residues. It is also possible that one or more of the disaccharide units are linked in a linear array to a second site. LPG is the first reported occurrence of an analogous lipid anchoring a polysaccharide. In fact, the leishmania parasite may be the first reported to use different glycolipids to anchor two distinct surface macromolecules (LPG and the promastigote surface protease often known as PG 63).

Amastigotes of leishmania are also reported to express LPG, although it is not clear which of the two forms of parasite produces it more efficiently. LPG is also reported to be present on the cell surface of leishmania-infected macrophages but it does not appear until at least six hours post-infection implying that it has an intracellular origin.

When the parasite burden is overwhelming, LPG accumulation ceases on the surface and it is either shed or internalised. One potentially important observation concerning LPG is its release from the surface of promastigotes and its appearance in the culture medium. The released LPG occurs in two distinct forms. One form binds very tightly to albumin in the medium and appears to be similar in structure to cellular LPG. Probably the lipid domain of LPG interacts with the hydrophilic form called 'phosphoglycan' (PG) but the significance of LPG conversion to PG has not been elucidated.

Immunological Aspects

The highly immunogenic nature of LPG is easily accounted for by its unusual structural features and as such a number of monoclonal and polyclonal antibodies have been generated against this heterogenous glycoconjugate. As an antigen, LPG is useful for serotyping leishmania strains, and immunization with LPG was found to protect mice against cutaneous leishmaniasis, suggesting the possibilities of molecularly defined leishmania vaccine. Incorporation of LPG into liposomes was significantly more effective than the use of LPG alone.

Functional Aspects

Some of the functions of LPG are believed to involve entry and attachment of the parasite to the host cell, whereas others are concerned with the survival of the parasite in the phagolysosomal system of the host. It promotes attachment of promastigotes to macrophages via activation and deposition of C3 receptors.

When the parasite enters the macrophages, it is open to attack from the cytocidal activities following the oxidative burst and action of the enzymes. LPG has a direct inhibitory effect on lysosomal enzymes and one such hydrolytic enzyme is beta-galactosidase.

Polymerase Chain Reaction : Non-Invasive Modality for Diagnosis, Prognosis and Treatment in Kala-Azar

In the eradication programme of VL, the thrust areas are : (1) the development of non-invasive, sensitive and specific test : (*a*) for diagnosis and prognostic evaluation of the disease, (*b*) identification of the carrier (2) development and evaluation of the efficacy of oral chemotherapy with drug combinations (3) defining objectively, clinical and parasitological cure in VL so as to prevent relapse, development of drug resistance and to prevent further transmission of the disease.

Conventionally, diagnosis of VL is based upon direct demonstration or culture of the parasite in samples aspirated from the bone marrow, spleen, lymph nodes or liver of infected patients. These procedures are non-invasive but necessitate the effort of experienced personnel and are associated with complications like infections and haemorrhage. In serological diagnosis of VL, immunofluorescence Assay (IFA), Enzyme Linked Immunosorbent Assay (ELISA) and Direct Agglutination Test have shown variable sensitivity (41-100%) and specificity. False positive reactions are common with other endemic diseases especially malaria, leprosy and tuberculosis. However, detection of circulating antigen in VL patients by specific monoclonal antibody has shown high sensitivity and specificity in certain studies.

PCR in the diagnosis of VL : In the genomic diagnosis of VL, DNA-DNA hybridization and PCR using kinetoplast DNA (K-DNA) as primers have shown promising results. The kinetoplast of leishmania is a unique DNA containing structure in the mitochondria of the cell. Kinetoplast DNA (K-DNA) comprises of two components : maxicircle and minicircle K-DNA. Minicircles are usually 1 kb in length, have no known function and are present in 10,000 - 20,000 copies. A comparison of the sequence of minicircles from different leishmania species has revealed that there is a

region of approx. 200 base pairs (bp) which is conserved between species. These characteristics have allowed their use as diagnostic probes. The sensitivity of DNA probes has been exponential through the use of amplification technology such as PCR.

In Kala-azar the infectivity of peripheral blood meal to the sandfly suggests that amastigotes must be circulating within the mononuclear cells in peripheral circulation. The number of these amastigotes must be very small as is evident by the fact that stained peripheral smears, thick or thin, are invariably negative for amastigotes in VL and leishmania carriers. We would like to hypothesize that PCR being a highly sensitive and specific technique, will be able to detect from carriers and patients, minutesimal quantity of *L. donovani* specific nucleic acid from peripheral blood buffy coat samples rich in monocnuclear cells.

PCR as a prognostic tool would be advantageous over those of bone marrow and splenic aspiration techniques as this is non-invasive, easy to perform and suitable for epidemiological studies. Intermittent evaluation of patients under various treatment schedules and for emergence of drug resistance studies through PCR will be more advantageous. By demonstration of presence or absence of parasite DNA in buffy coat preparations, the parasitic cure of VL can be conveniently monitored and correlated with clinical cure. This would be a more acceptable approach than repeat bone marrow or splenic puncture examinations. It would thus allow to define clinical and prarsitological cure of VL and help to develop and standardize new oral treatment with a view to stop antimonial injections for a period of 3 to 4 weeks.

The objectives of the PCR non-invasive modality are :

(*i*) To purify kinetoplast DNA (K-DNA) from the promastigotes of *Leishmania donovani*, prepare the K-DNA probes by nick translation and standardized Dot blot DNA-DNA hybridization assay using these probes in the diagnosis of visceral leishmaniasis.

(*ii*) To develop and standardize polymerase chain reaction from buffy coat and bonemarrow aspirates in visceral leishmaniasis.

(*iii*) To evaluate the sensitivity, specificity and predictive value of PCR with that of conventional methods e.g. microscopy, culture and serology, used in diagnosis and prognostic evaluation of visceral leishmaniasis.

(*iv*) To evaluate a combination of allopurinol 20 mg/kg/day and ketoconazole 600 mg/day as first line treatment for visceral leishmaniasis.

(*v*) To evaluate liposomal amphotericin B for treatment of drug resistant Kala-azar.

(*vi*) To standardize the criteria for duration of therapy, drug response and drug resistance in Kala-azar utilising the established and newer diagnostic methods.

CHEMOTHERAPY IN KALA-AZAR

After continued successful therapy in visceral leishmaniasis for years by exhibiting urea stibamine discovered by Sir U.N. Brahmachari in 1922, it became apparent that although the pentavalent form of anti-

mony was more effective when given intravenously, it was certainly more toxic than the same drug (sodium antimony gluconate) when given by the i.m route as the toxic reactions were not frequently fatal. Amazingly there was hardly any recurrence or relapse and children tolerated sodium stibogluconate (stibanate) very well when it was given daily by the intramuscular route in a dose of 20 mg/kg of body weight for 20 to 30 days, thus saving millions of lives in the bygone years. But now the percentage of resistant cases has increased from 5 to 25% and there is a continued search for newer drugs like hydroxystilbamidine isethionate (lomidine) and pentamidine isethionate in a dose of 3 to 4 mg/kg of body weight but the latter drug can cause irreversible diabetes mellitus and also anaphylactic reaction in a small number of cases.The drug of choice now in antimony resistant cases is amphotericin B, an antifungal drug used successfully in the past in patients with tubercular cavitation and superimposed with fungal infection. The initial dose is 0.25 mg/kg which should be gradually increased to 1 mg/kg and adiministered in a 5% solution of dextrose over a period of 4 to 6 hours. In our first case of leishmanial meningitis, this practice was followed for a period of three weeks for successful eradication of the neurological complications in a resistant case of Kala-azar. The modern trend, however, is to use amphotericin B suspended in lipomatous solution as it can be injected more rapidly than in 5% glucose solution.

Another antifungal drug known as ketoconazole has been used by the author in a number of Kala-azar cases resistant to conventional treatment in the pediatric age group. The results of treatment in about two dozen resistant cases in the age group of 5-20 years have proved very successful when given orally in the dose of 200-400 mg daily. It also has the merit of crossing the blood-brain barrier as it proved effective in generlised epileptic form of convulsions caused by *Leishmania donovani* in a case of PKDL in a boy of 20 years.

Conclusion

A detailed clinical, pathological and immunological survey has been made in the above article with an attempt to focus the importance of lipophosphoglycan (LPG) in visceral leishmaniasis.

Two cases of insult to the central nervous system by *Leishmania donovani* have been included (meningeal and cerebral) in this article. It is noteworthy that this has not been documented before by any other scientific worker.

Even with full knowledge of Kala-azar in all its aspects, the disease has not been eradicated uptil now.

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EVIDENCE-BASED MEDICINE

"Practice evidence-based medicine; it ensures optimal patient care".

This was the message of the FOGSI-Rallis oration delivered by Dr. J. Evers, Prof. of Obstetrics & Gynaecology, and Director of Reproductive Endocrinology and Fertility, University of Maastricht, Holland, at the 42nd All India Congress of Obstetrics and Gynaecology in Hyderabad recently.

Evidence-based medicine is defined as "the conscientious, explicit and judicious use of current best evidence in making decisions about the care of individual patients."Dr.Evars stressed that research papers appearing even in the highly respected journals should be evaluated critically since what is read may be true for a big tertiary care centre, but it may not be true in the regular day-to-day clinic. Explaining about proper evaluation of an article, he emphasized on 3 points :

(1) A paper on a new diagnostic test must carry a comparison with the gold standard
(2) A paper about prognosis must include follow-up data on cohort groups
(3) A treatment study is valid only if it is a randomised clinical trial.

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