Post Kala-azar Dermal Leishmaniasis (PKDL)

In vivo veritas

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· WEDNESDAY, NOVEMBER 2, 2011 ·

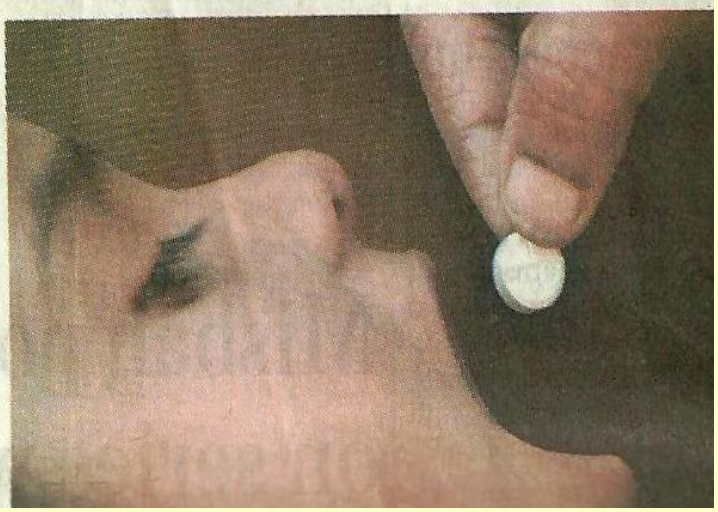
THE HINDU

INDIA'S NATIONAL NEWSPAPER SINCE 1878

India facing heavy burden of neglected tropical diseases

Narayan Lakshman

WASHINGTON: Even as the world welcomed the seven billionth member of the global population this week, medical researchers warned that rapid-growth economies such as India still had a high proportion of morbidity, with more than 290 million Indians suffering from Neglected Tropical Diseases (NTDs).



These NTDs include visceral leishmaniasis, also known as "kala-azar"; lymphatic filariasis which causes elephantiasis; leprosy; dengue fever; rabies; and soil transmitted helminth.

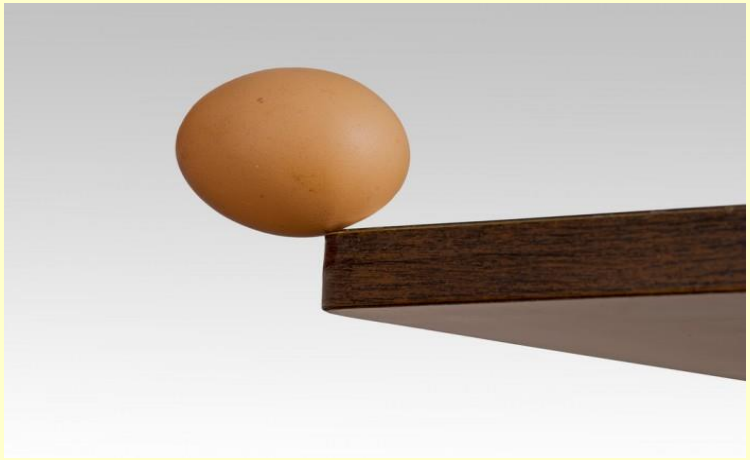
ASIA TIMES

Killer kala-azar dropping below tipping point

Incidence of black fever (called 'kala-azar') in the Indian subcontinent is declining fast – and dropping below the tipping point – thanks to the interventions of World Health Organization (WHO) and the combined efforts of governments. However, fears of its resurgence are haunting the.. ***click here to read more***

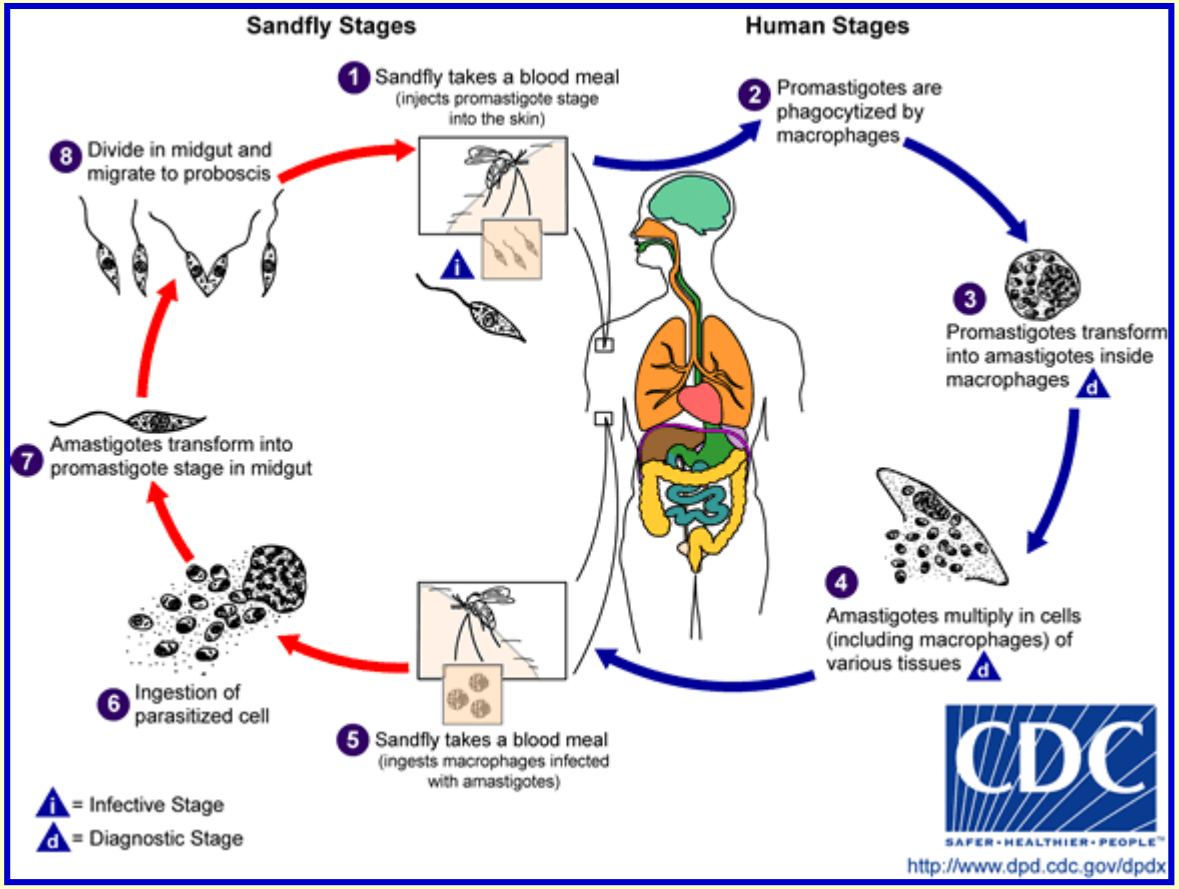
26th August, 2016

1st February 2017; Health Budget highlights.....



- **2017: Elimination of Kala Azar & Filariasis**
- **2017: Elimination of Leprosy**
- **2020: Elimination of Measles**
- **2025: Elimination of TB**

What is Kala-azar/Visceral Leishmaniasis?

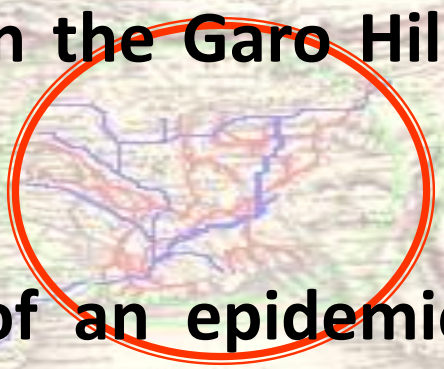


"The longer you can look back, the further you can look forward"

Winston Churchill (1944)

Enemy Unknown

- 1880s: A mysterious fever started in the Garo Hills of Assam

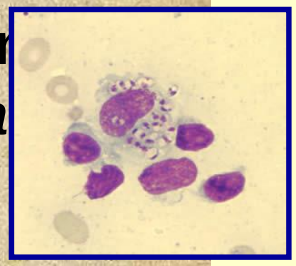


- 1890s: The fever takes the form of an epidemic: "Quinine resistant" malaria appears in Dumdum, Burdwan etc.

- 1900: The epidemic spreads to most of India, especially the East and South

Enemy identified

1903: William Leishman: Prof. of Pathology in ar autopsy identified amastigote forms of *Leishma donovani*.



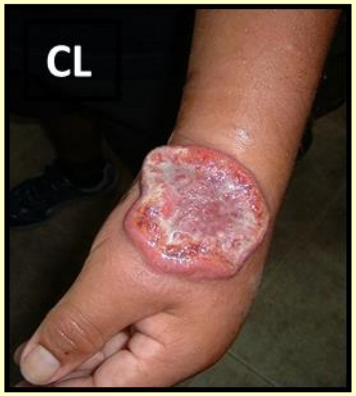
1903, Charles Donovan, Professor of Physiology, Madras University, sent a sketch of parasites (in the spleen of a patient suffering from spleen enlargement and fever) to Sir Ronald Ross.

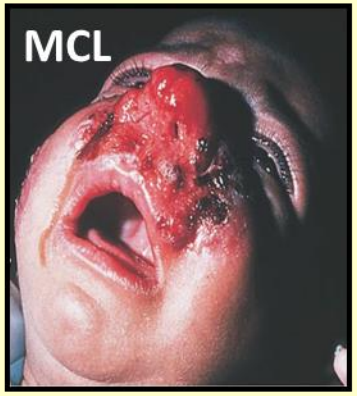
Ross labeled them as "Leishman-Donovan" bodies

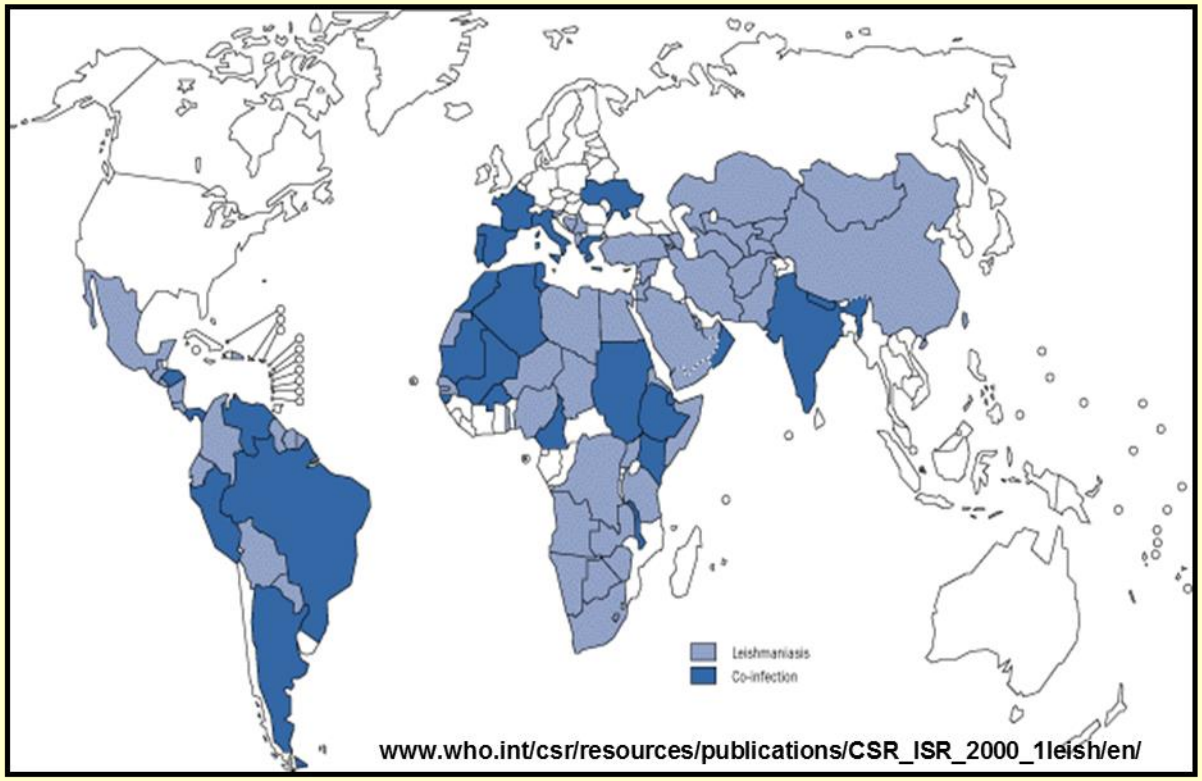
The Royal Victoria Hospital, Netley, UK

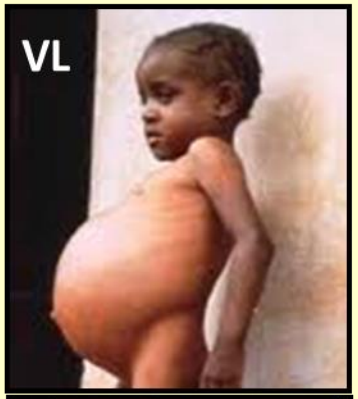
The Royal Victoria Hospital, Netley (by permission of the *Illustrated London News*).

Leishmaniasis: A global burden



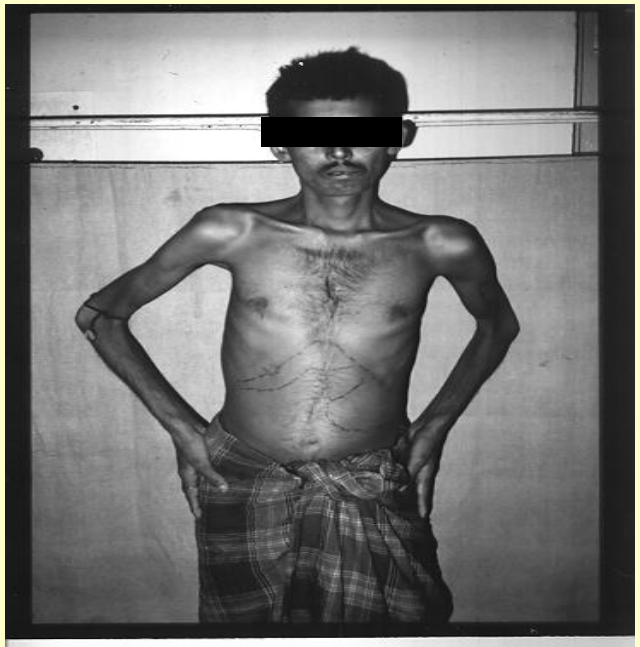


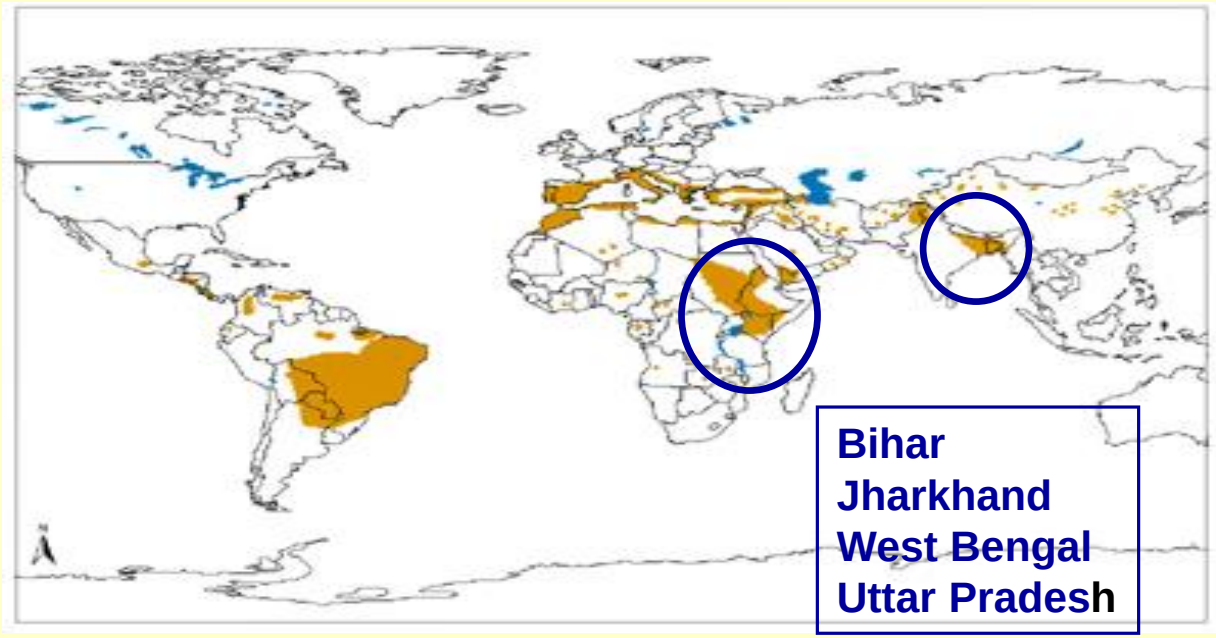


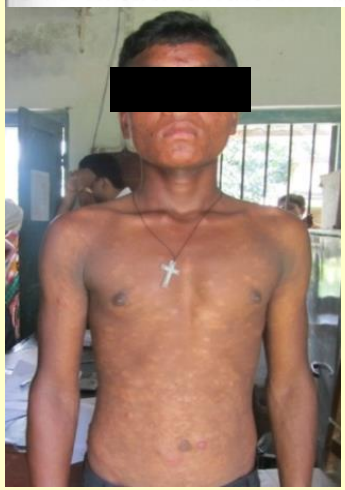


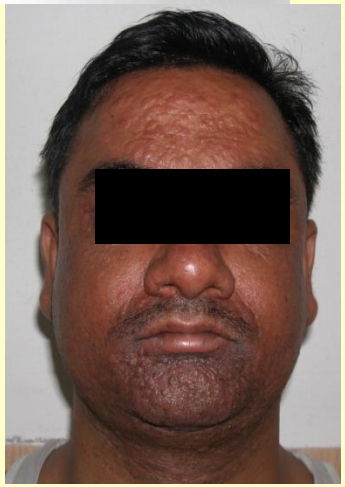
- A total of 98 countries in 5 continents reported endemic Leishmaniasis
- Official case counts totalled more than 58,000 VL cases and 220,000 CL cases per year
- More than 90% of global VL cases occur in six countries: India, Bangladesh, Sudan, South Sudan, Brazil and Ethiopia
- Tentative estimate of 20,000 to 40,000 leishmaniasis deaths per year (mainly of VL)

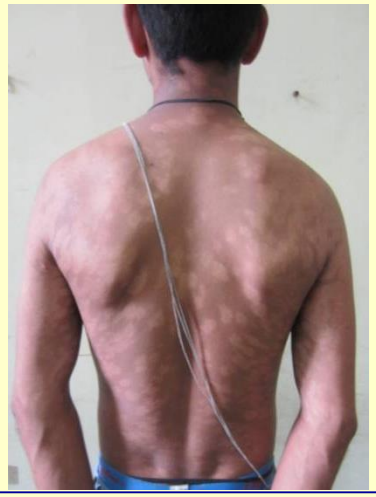
Visceral Leishmaniasis has a more limited geographical distribution
Post kala-azar dermal Leishmaniasis, PKDL, the sequel to VL

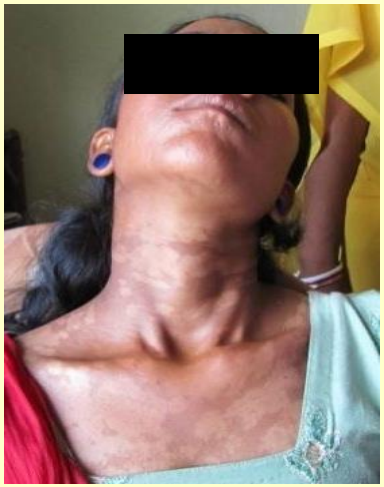












Narrow geographical spread, elimination/eradication is feasible

A series of delayed targets in the elimination programme

70.7% decline in cases & 80.5% decline in deaths.

Pentalateral MOU (India, Nepal, Bangladesh, Bhutan & Thailand.

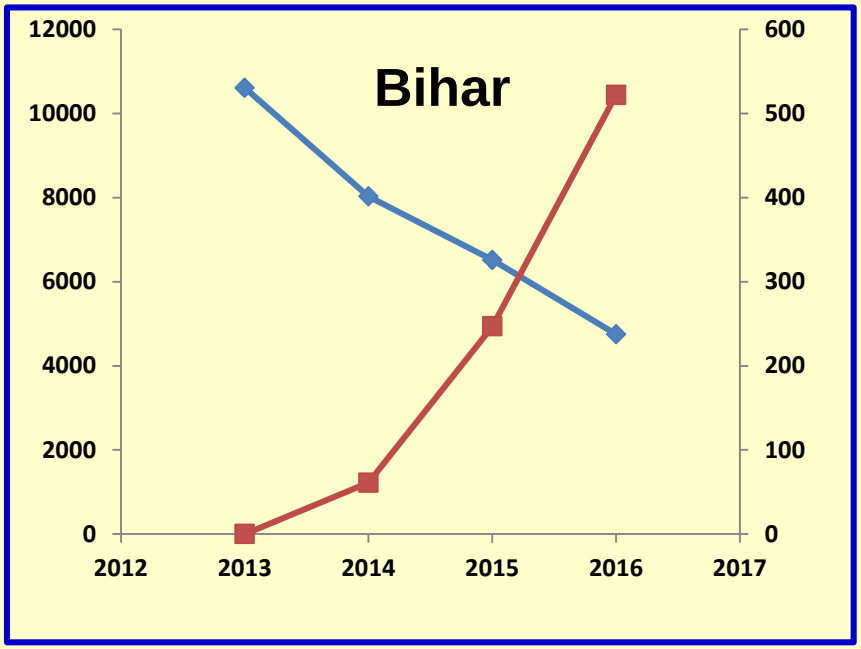
1 case per 10,000/zilla/year by 2015

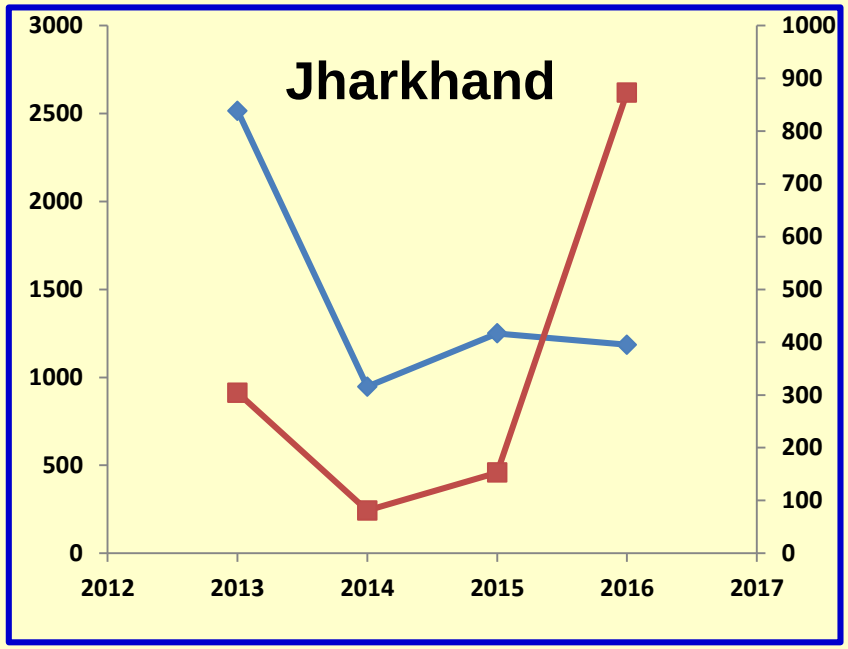
Emergence of SAG Resistance

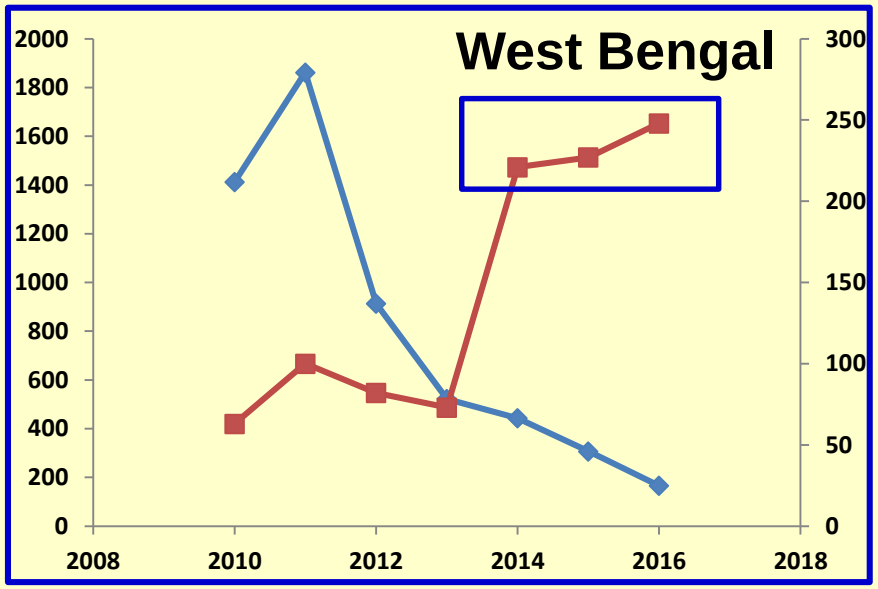
Active surveillance
Identification of PKDL as disease reservoir

London declaration: control of schistosomiasis, soil-transmitted helminthes, Chagas disease, visceral leishmaniasis and river blindness.

Active surveillance: a game changer.....



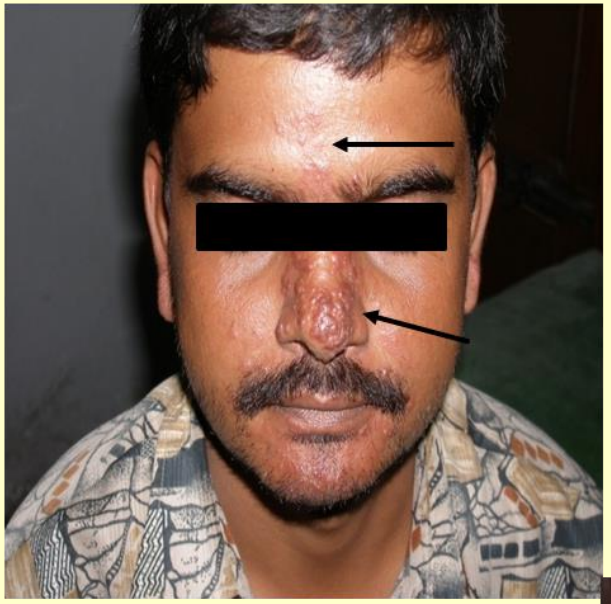
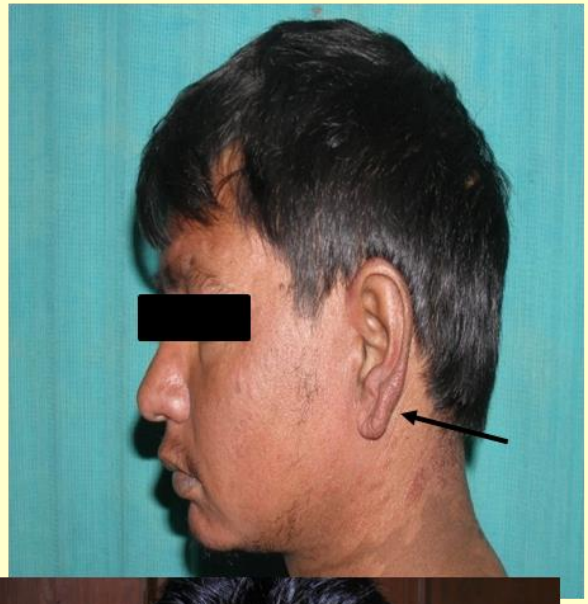
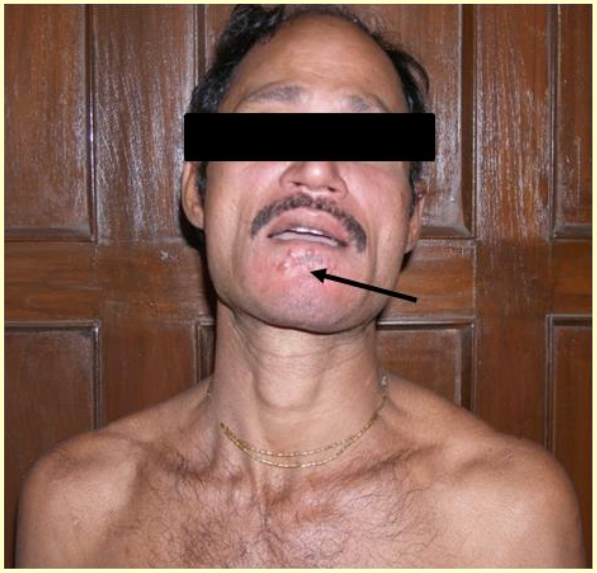
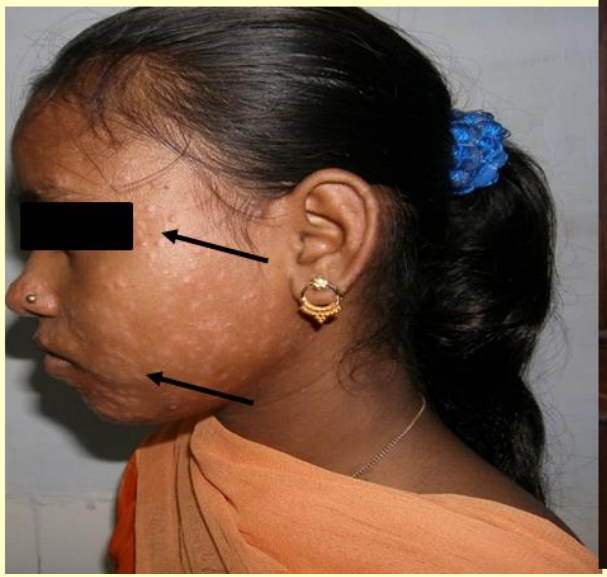
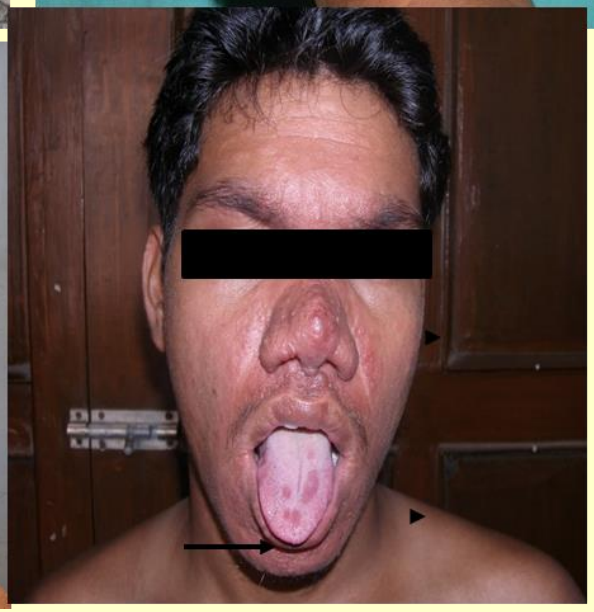




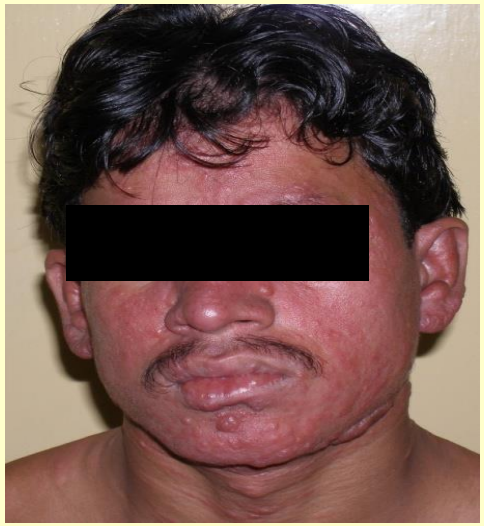
VL

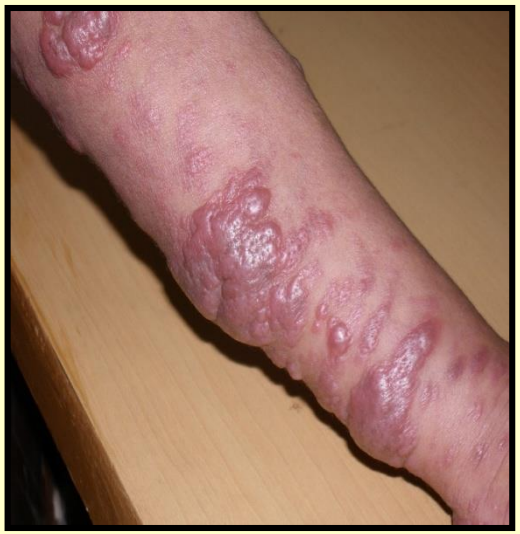
PKDL

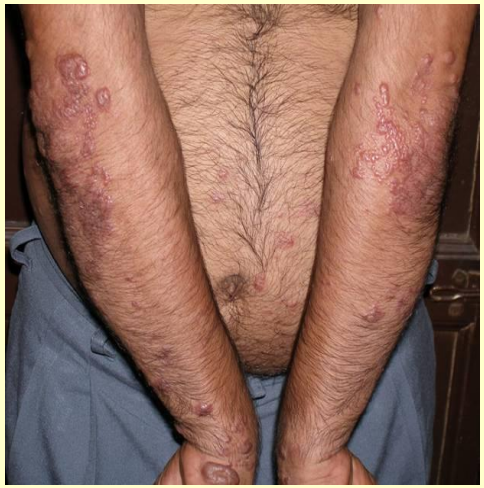
Dermal lesions are rich in parasites;
Patients with PKDL are considered as the disease reservoir

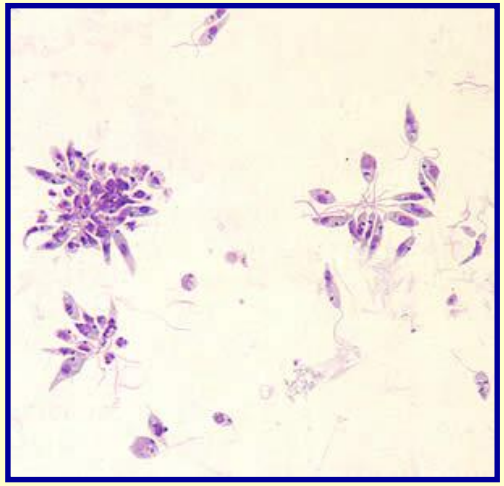







Identifying PKDL as the disease reservoir









Xenodiagnosis

qPCR of skin biopsies from 3 patients with maculopapular or nodular (PKDL):

Parasite load: 1428-63058/ µg DNA

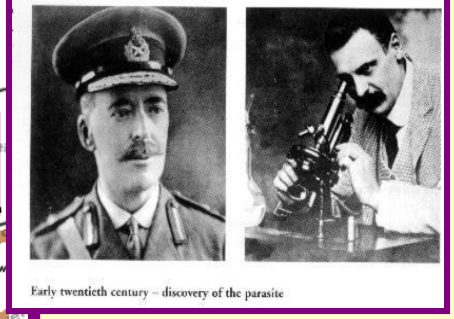
Molina et al. Clin Infect Dis. 2017;
doi: 10.1093/cid/cix245

Elimination is achievable…….

- Man is the only host
- Sandfly is the only vector
- Rapid diagnostic tests exist (PKDL?)
- Effective drugs are available (PKDL?)
- Geographical spread is limited to 54 districts
- High political commitment

PKDL, the silent disease reservoir


N
Vivekanda Bridge
Bali
Bharahanagar
Hugli
Barakpur
Sindhi
Belur Math
Kasipur
Dumdum
Birla Planetarium
Ghusuri
Chitpur
Tata Park
Bamangachhi
Bagbazar
Shyam Bazar
Begachiya
Patipukar
Armenian Church
Jewish Synagogues
Shakiya
Shoba
Beniatola
Deshbandhu Park
Gobabari
Pareshnath Jain Temple
Shobha bazar
STM
Bantra
Howrah Bridge
Parsi Temple
Ghose Bagan
Barabazar
HARORA
Nakhoda Mosque
St. John's Church
B.B.D. Bazar
Eden Gr.
IPGMER
Andul Rd.
Shalimar
Museum
Hugli
Race Course
Bhawanipur
Beniapukur
Tanga
Garden reach
Polo Ground
St. Paul's Cathedral
Watganj
Rabindra
Park Circus Maidan
khidirpur
Bhawanipur
Mominpura
Alipur
Baliganj
Kustia
Horticultural Garden
Jatindas
Kasba
Dakshineswar Kali Temple
Kalighat
Chetla
Ramakrishna Mission Institute
Dhakuria
Tarattala
Rabindra Sarobar
Rabindra Sarobar
Rabindra Sarobar Stadium
Refugee Colony
Tali Ganj
TollyGunje
Golf Course
Refugee Colony
Railway Line
Major Station
Roads
River
Tourist Attraction
Kolkata Tourist Map
Map not to Scale
Copyright © Indian Holiday Pvt. Ltd. 2006-07


Early twentieth century – discovery of the parasite


Romsons

Immunopharmacology group, IPGMER, Kolkata











Chemotherapy

Immunopathology

Monitoring chemotherapy

Diagnosis

Challenges in research on PKDL

- No animal model exists
- Disease of the poorest of the poor
- Causes morbidity, but no mortality, and so PKDL patients do not actively seek treatment
- Parasites are limited to skin lesions; patients may not provide a skin biopsy
- As PKDL is a NTD, tough to lure the pharmaceutical industry

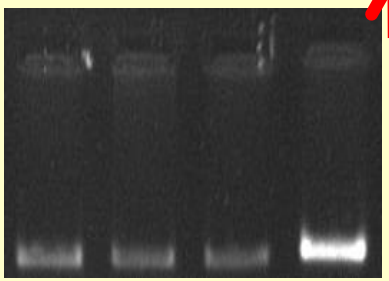
Since December 2014…..

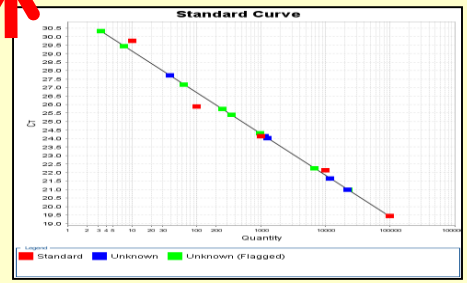
Door to door survey

KTS (Kala-azar Technical Supervisor), identify suspected cases of PKDL

Initiation and monitoring of Treatment

Cases report at the Medical Camps; our teams examine





ITS-1PCR and qPCR performed at IPGMER

Samples collected
(for PKDL, dermal biopsies, sent to IPGME&R)

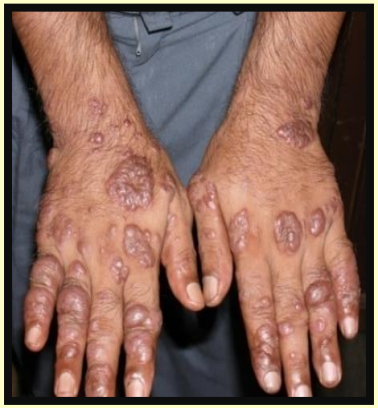
At ground zero……

Dr. Manab Ghosh, Dr. Surya Jyati Chowdhury, Dr. Bikash Sardar

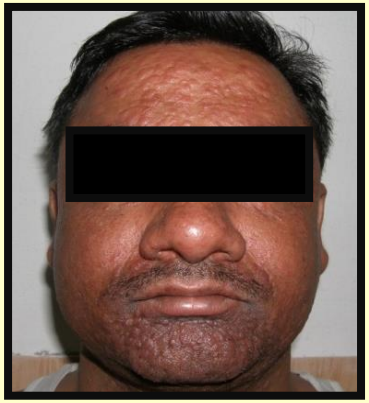
Laboratory Diagnosis of PKDL

Clinical Suspicion
History of VL

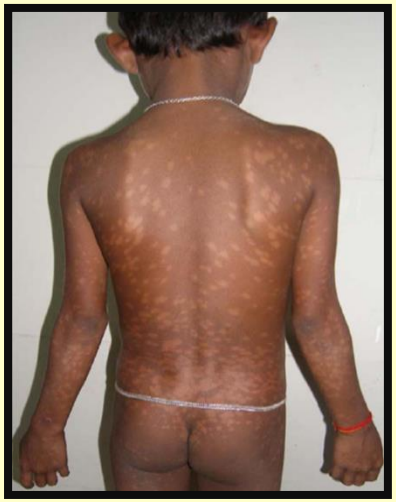
Nodules



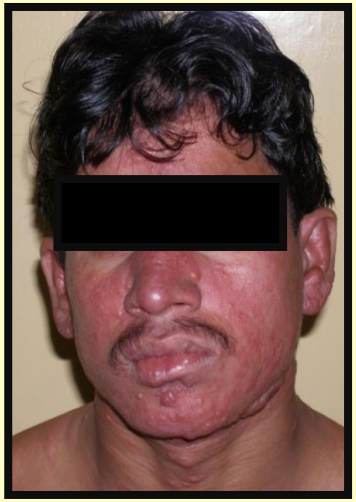
Papules



Macules

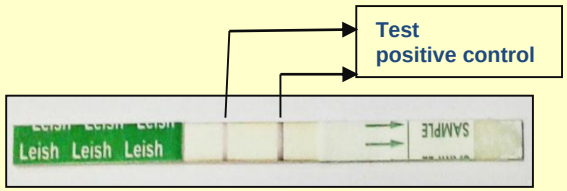


Polymorphic



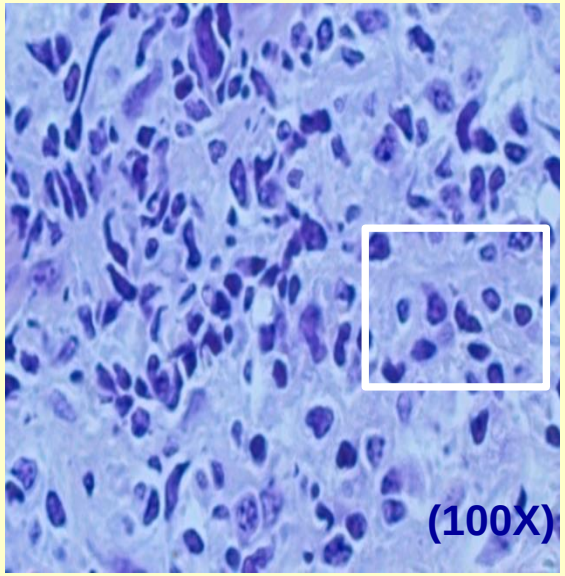
Antibody Based Detection

- ELISA
- rK39 strip test



Antigen Based Detection

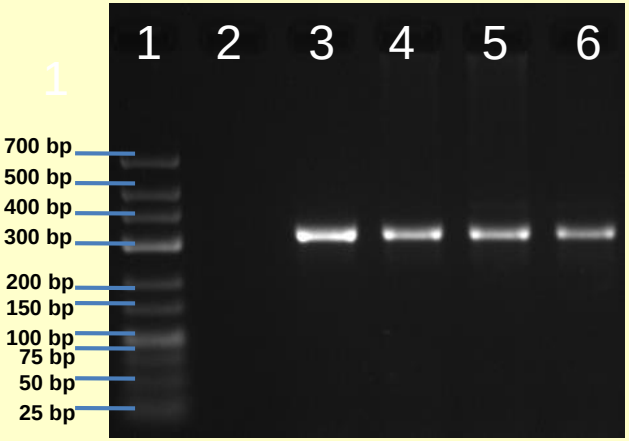
- Microscopy (LD bodies)
- Parasite Culture
- PCR



PCR based diagnostic approaches for PKDL

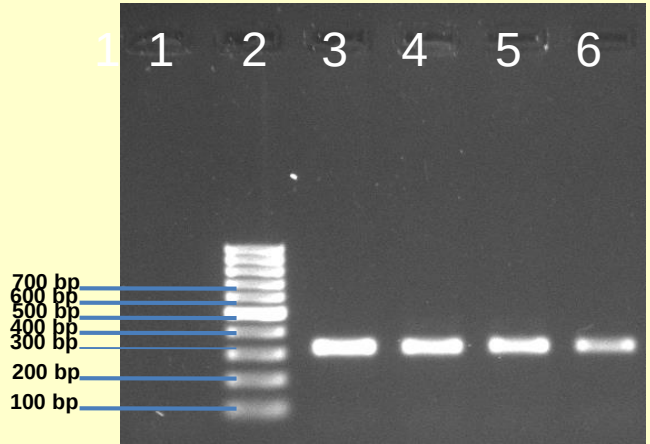
Qualitative: ITS-1 PCR

Visceral Leishmaniasis (VL); blood



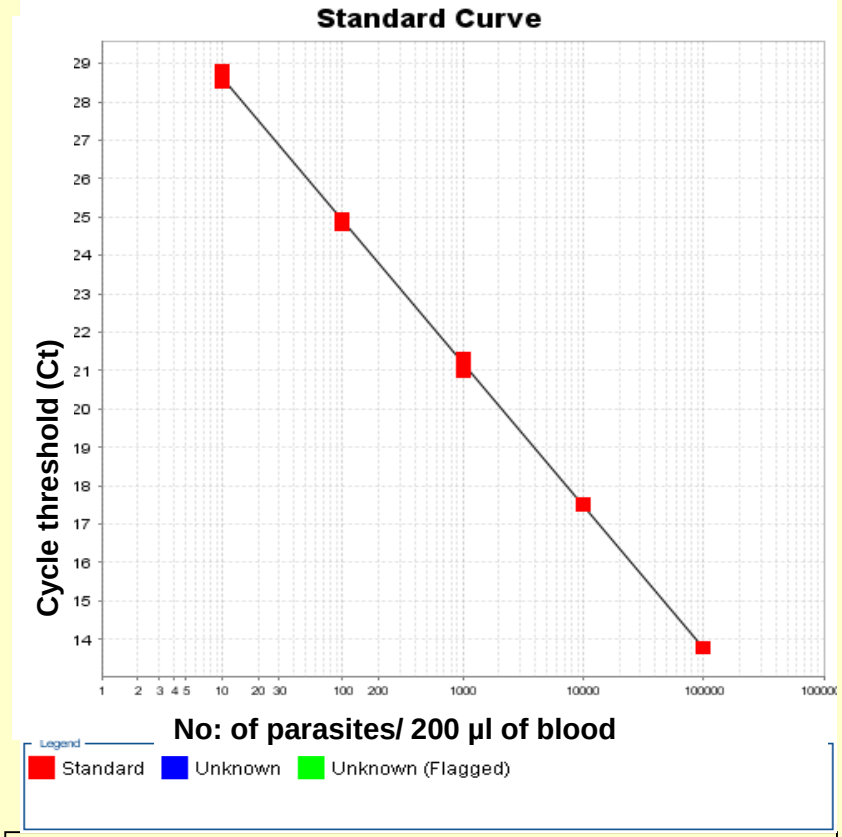
Lane 1: Marker (low m.w. ladder)
Lane 2: Blank
Lane 3:? VL
Lane 4:? VL
Lane 5:? VL
Lane 6 : AG 83 positive control

Post Kala Azar Dermal Leishmaniasis (PKDL); dermal biopsies



Lane 1:Blank
Lane 2:Marker (100bp ladder)
Lane 3:? PKDL
Lane 4:? PKDL
Lane 5:? PKDL
Lane 6 : AG 83, positive control

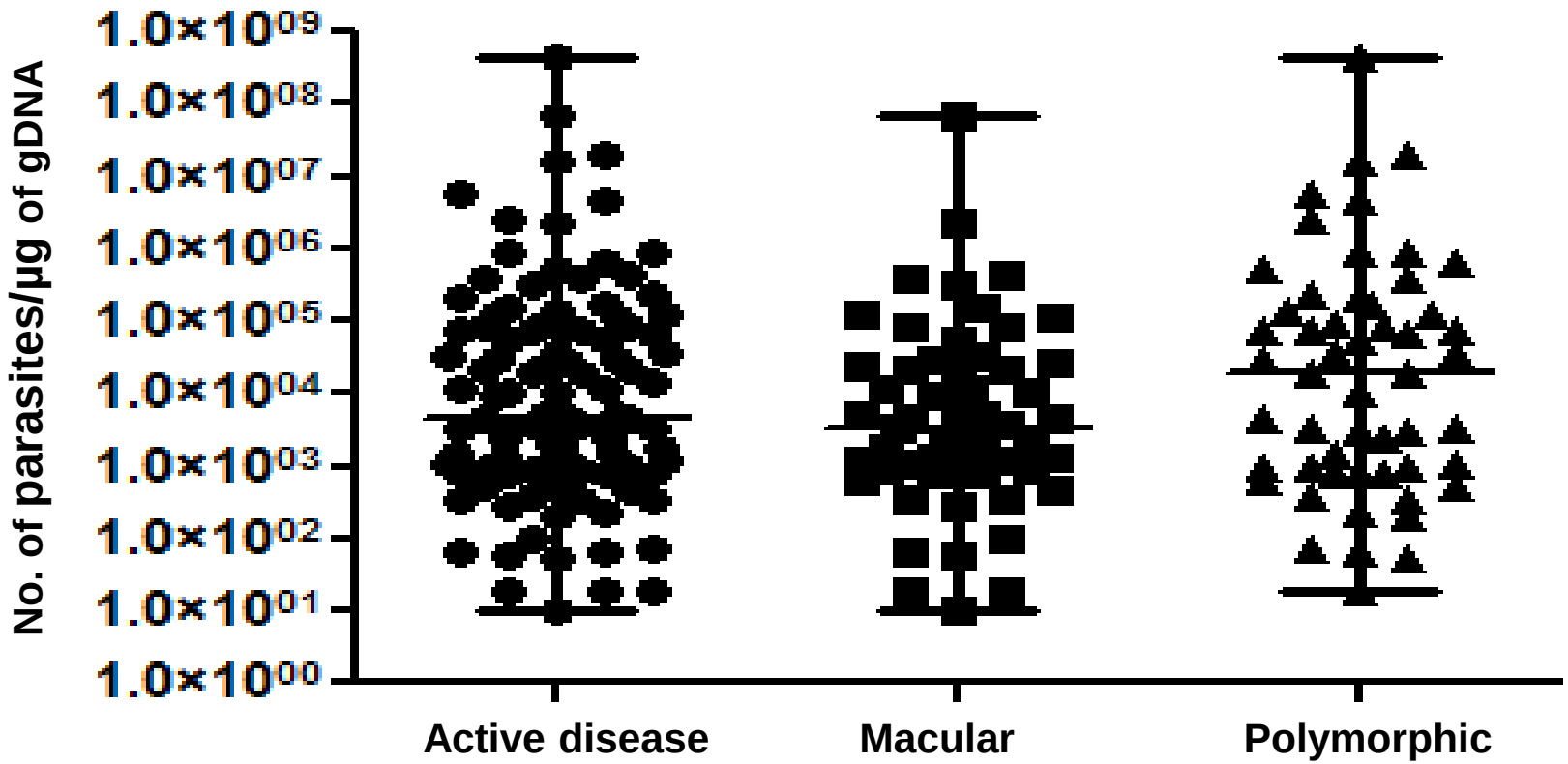
Quantitative:Real time PCR



Standard curve for estimation of number of *Leishmania* parasites(Ct = cycle threshold); performed in triplicates

Parasite Load: no: of parasites/µg of gDNA

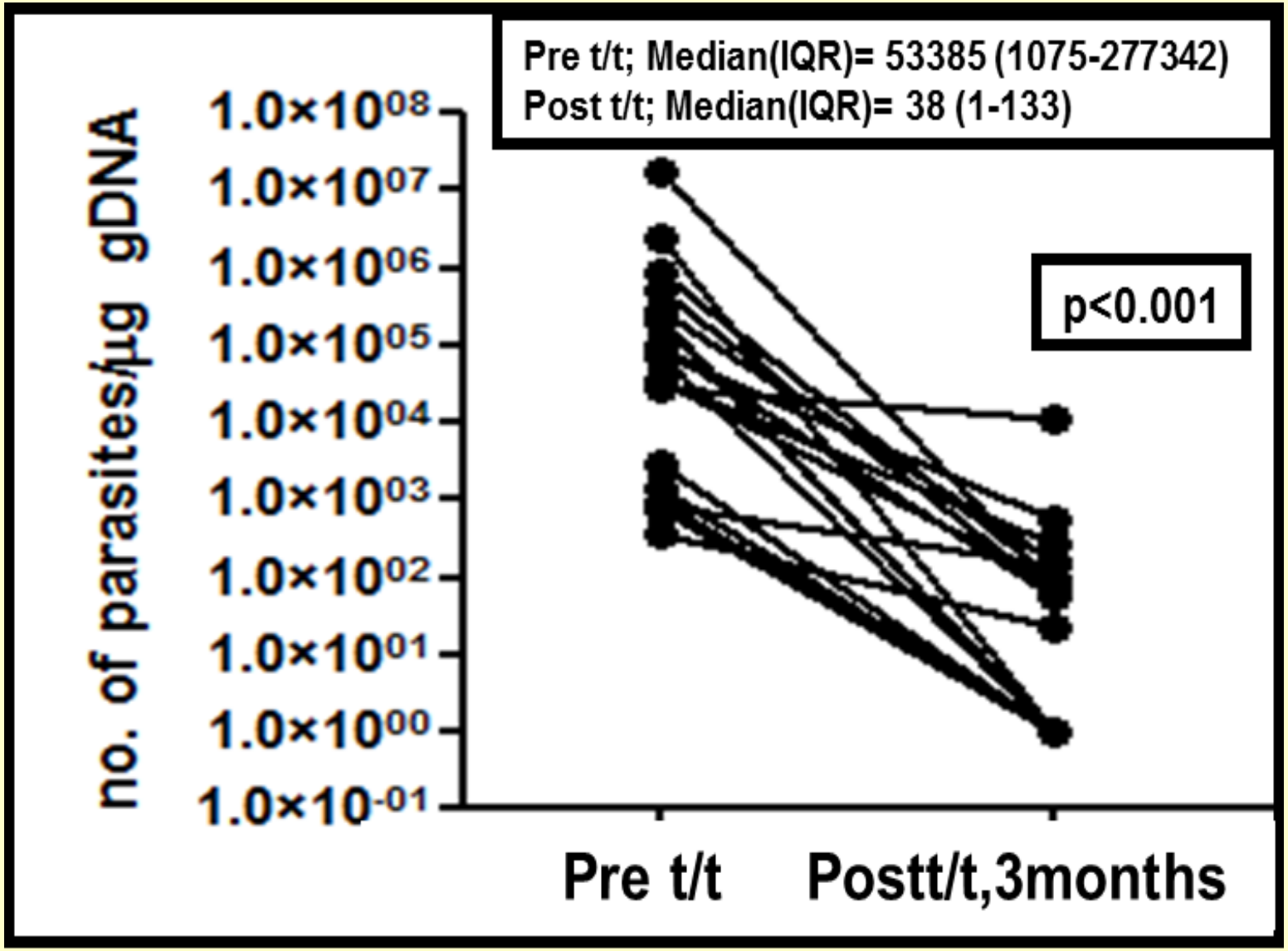
Distribution of PKDL (n = 109)



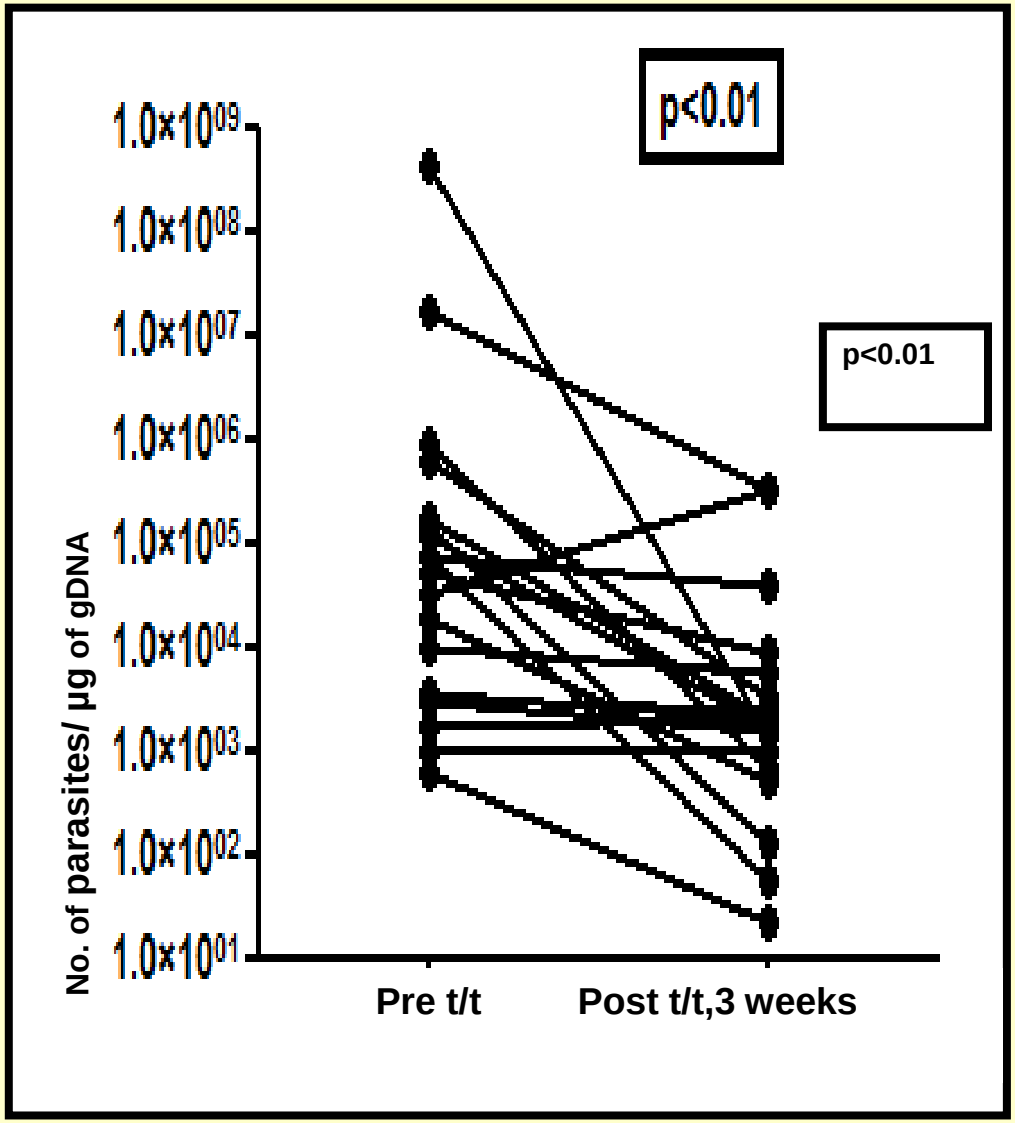
n = 109	n = 56	n = 53

PKDL (n=109) (●) Median (IQR) = 4377 (865-80441)
Macular PKDL (n=56) (■) Median (IQR) = 3366(857-25166)
Polymorphic (n=53) (▲) Median (IQR) = 18943 (854-186603).

Excellent clearance of parasites by Miltefosine

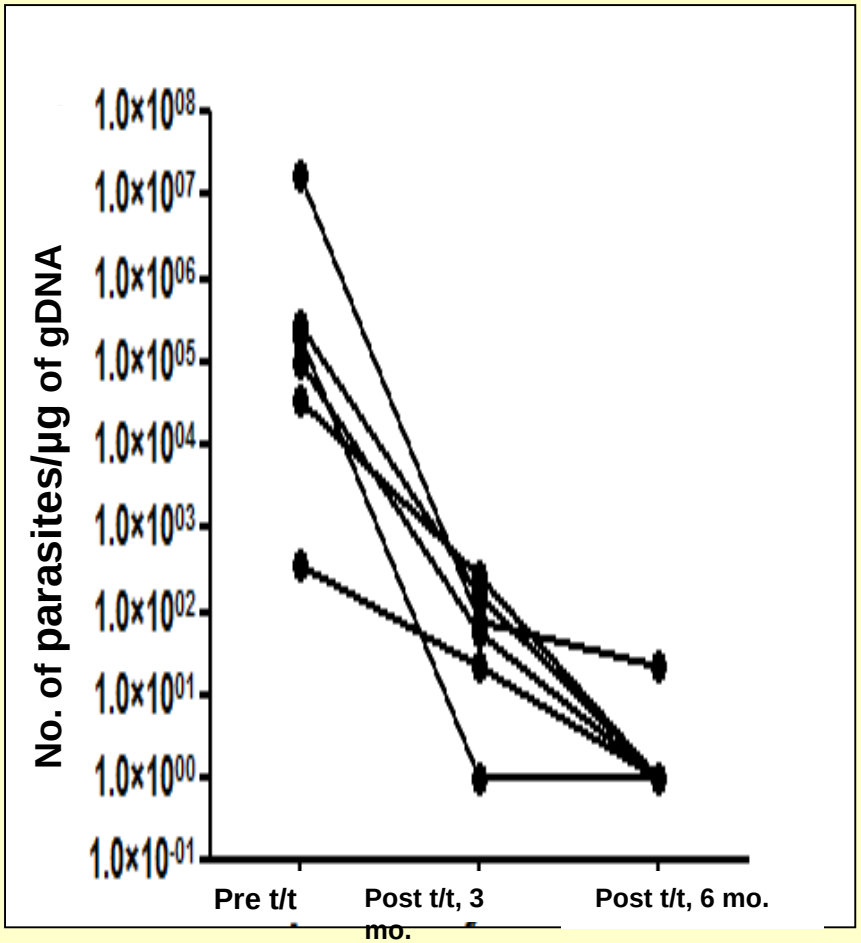


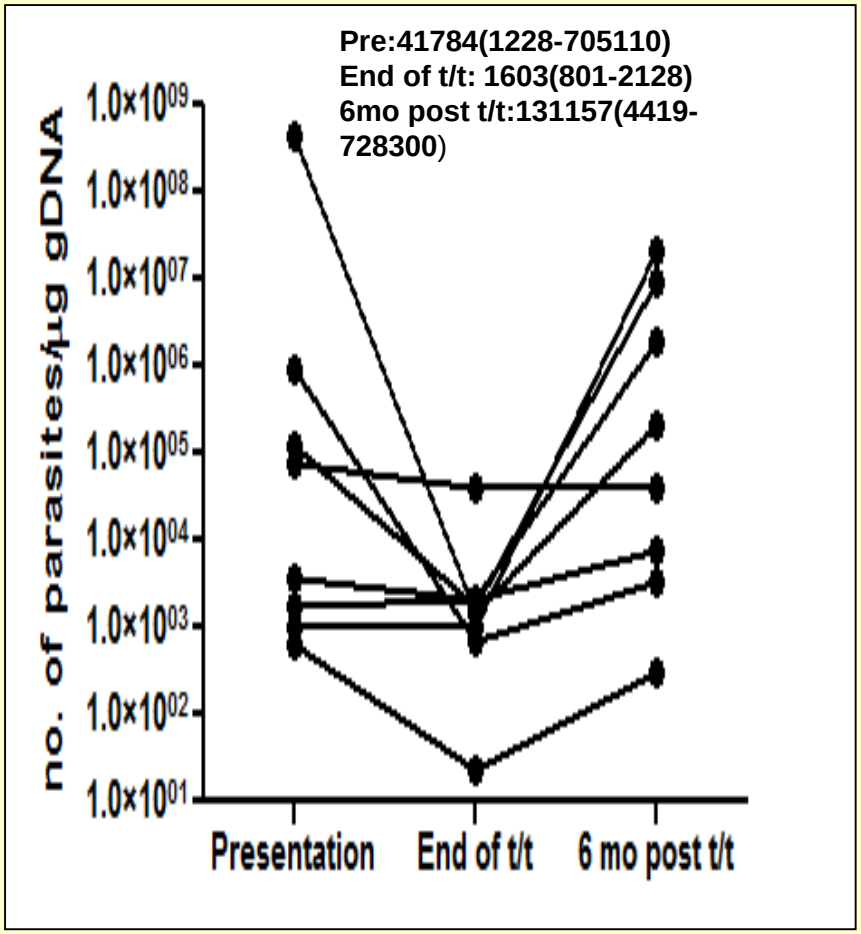
LAmB caused incomplete clearance of parasites



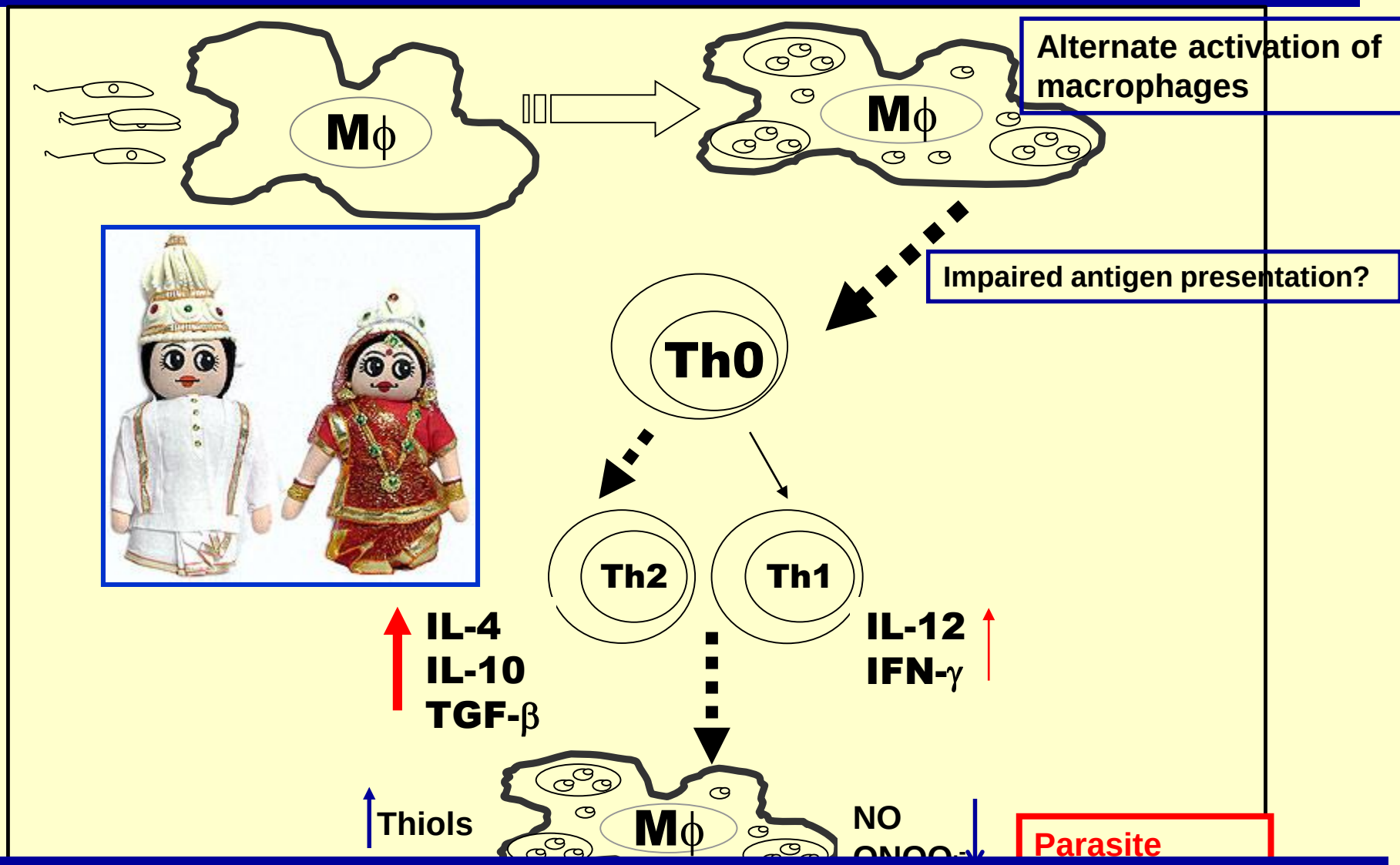
Pre t/t =51923 (3231-175844)
Post t/t= 2061 (731-5763)

6 months later.....



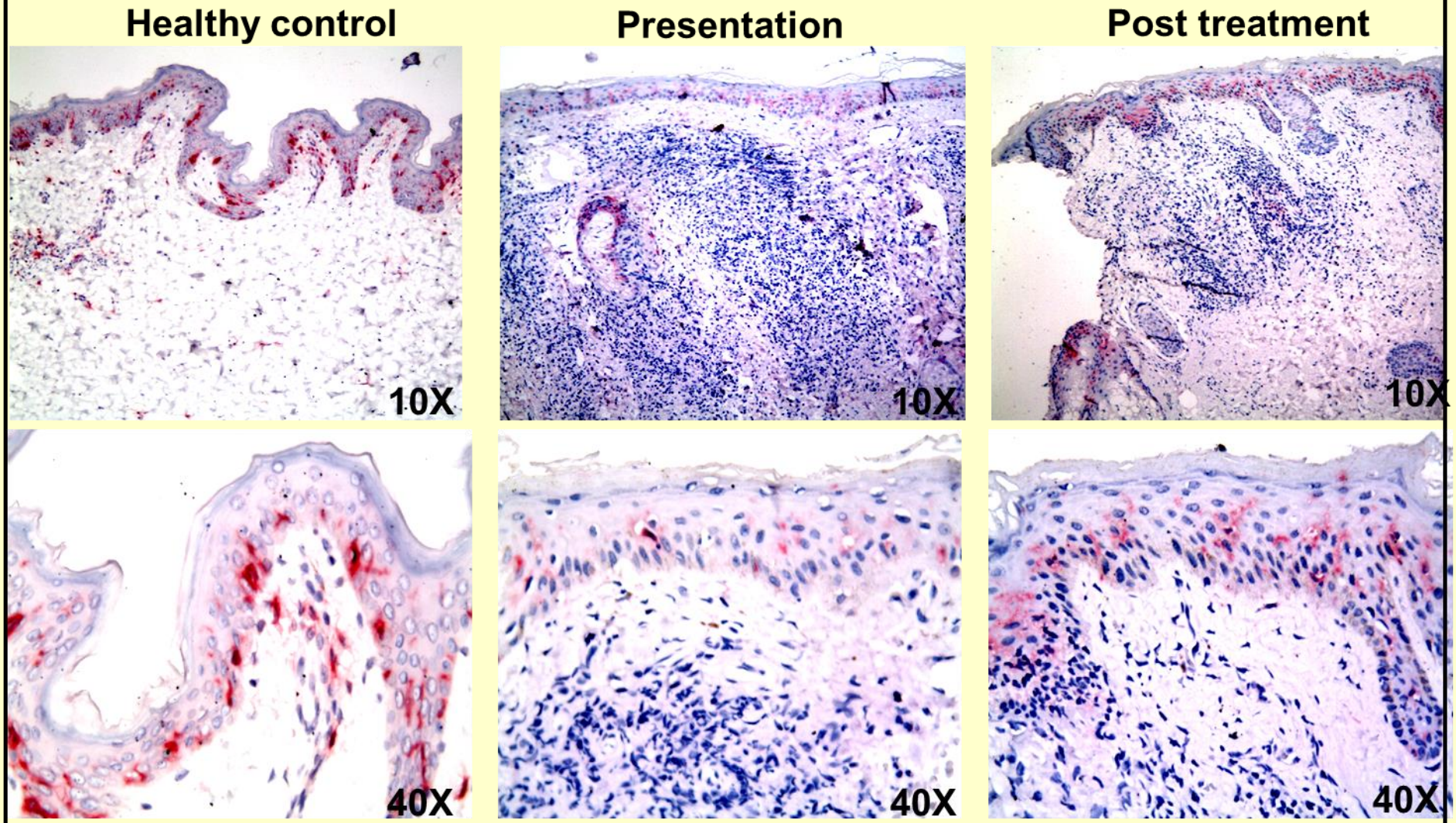


Leishmania weds macrophage; a marriage of convenience



In PKDL…..*in vivo veritas*?

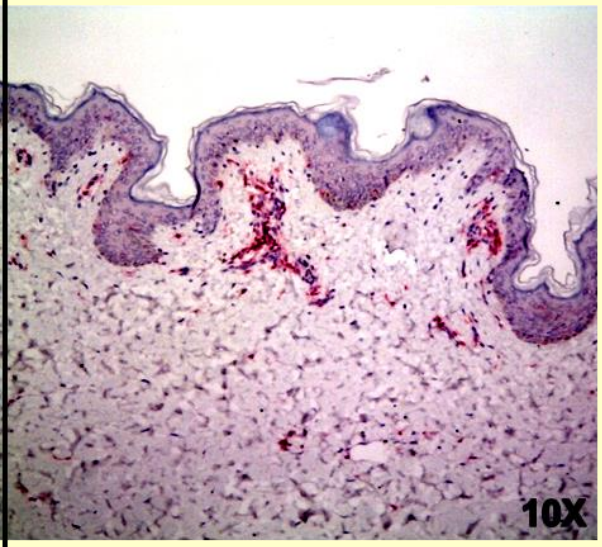
Decreased CD1a^{+} dendritic cells in PKDL



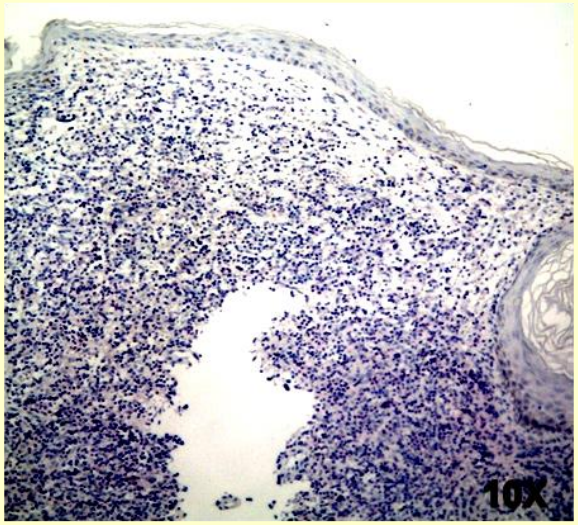
Mukherjee et al., Exp Dermatol. 2015; 24:232-4

Near total absence of $CD4^+$ T-cells

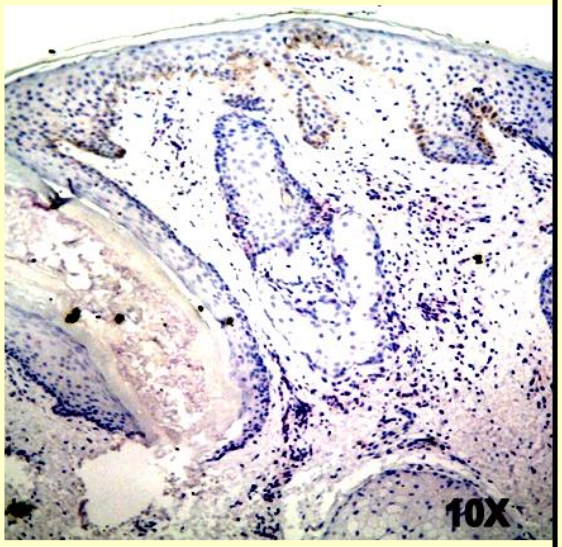
Healthy control



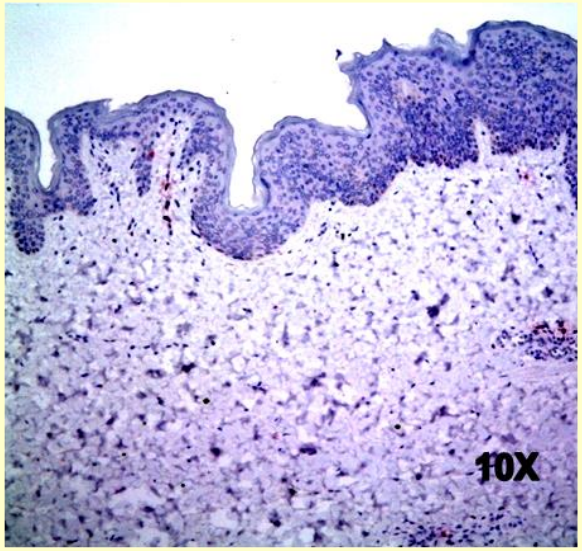
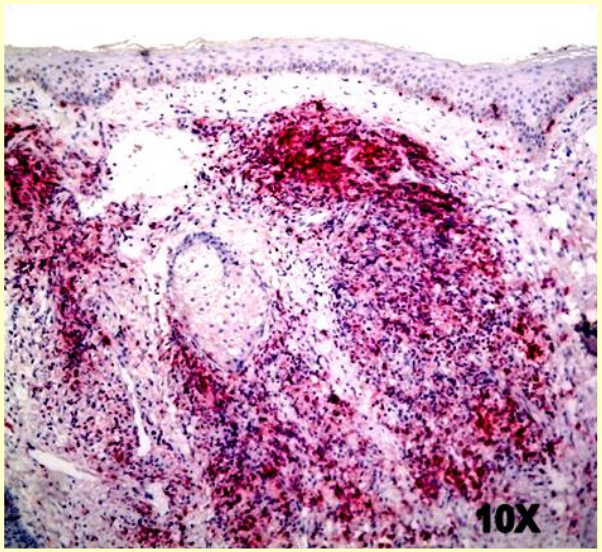
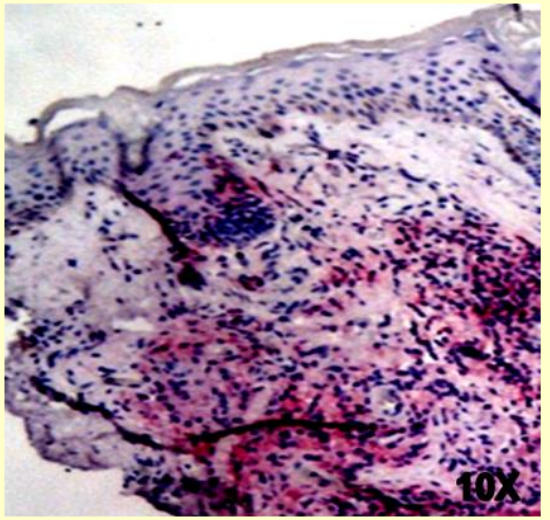
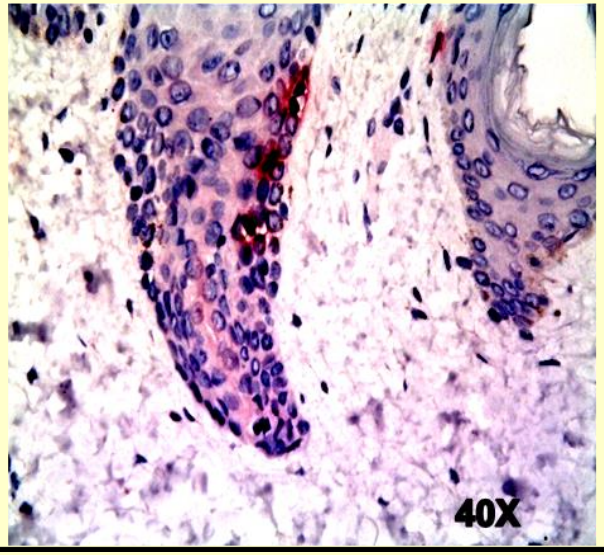
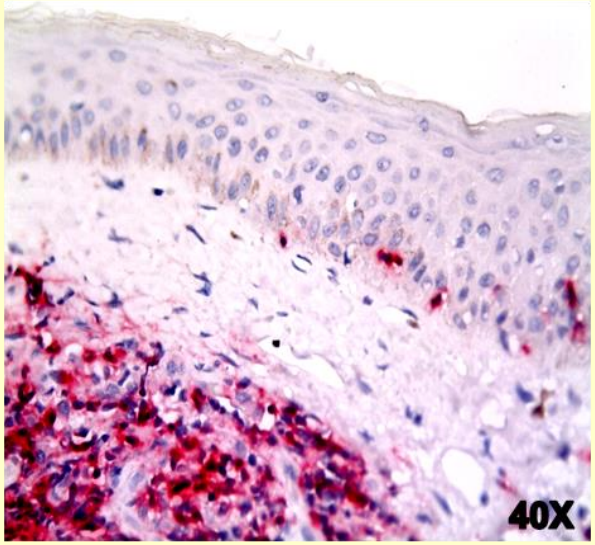
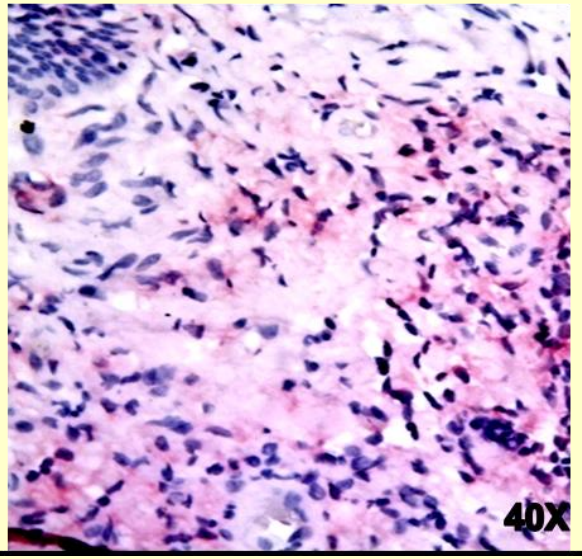
Presentation



Post Treatment

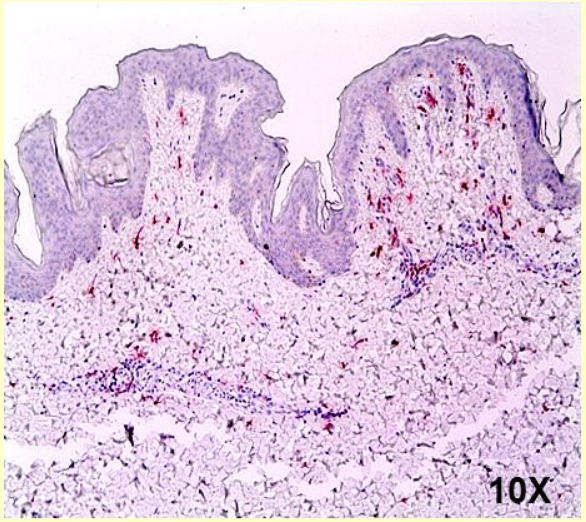


Heavy infiltration of $CD8^+$ T-cells

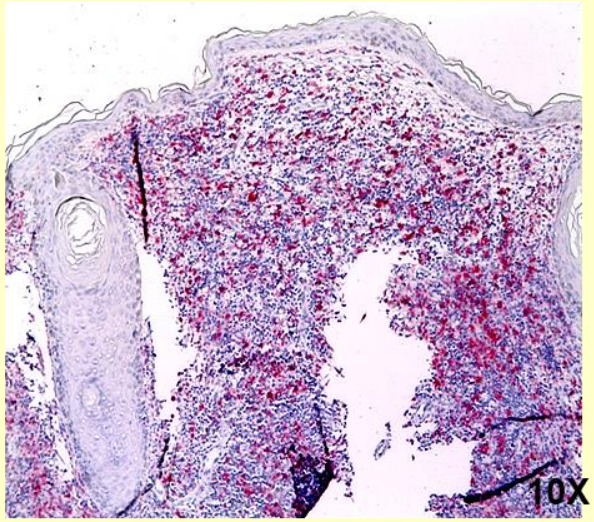
Healthy control	Presentation	Post Treatment
		
		

Increased CD68^{+} macrophages in PKDL

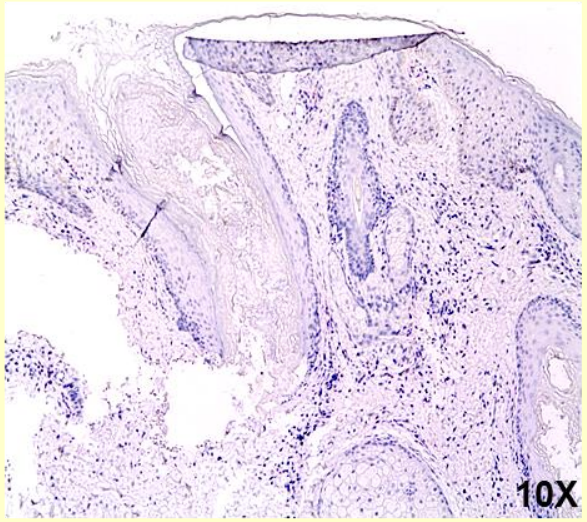
Healthy control

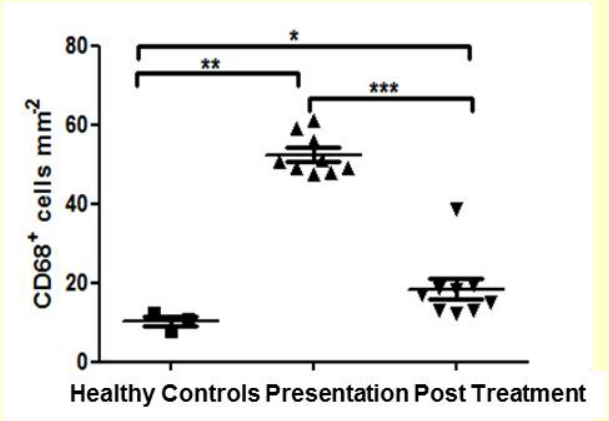


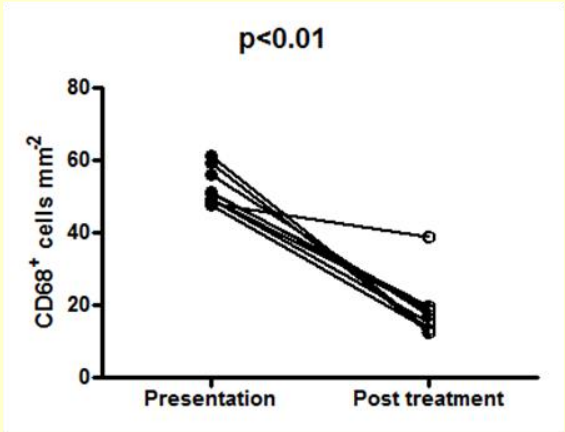
Presentation



Post treatment

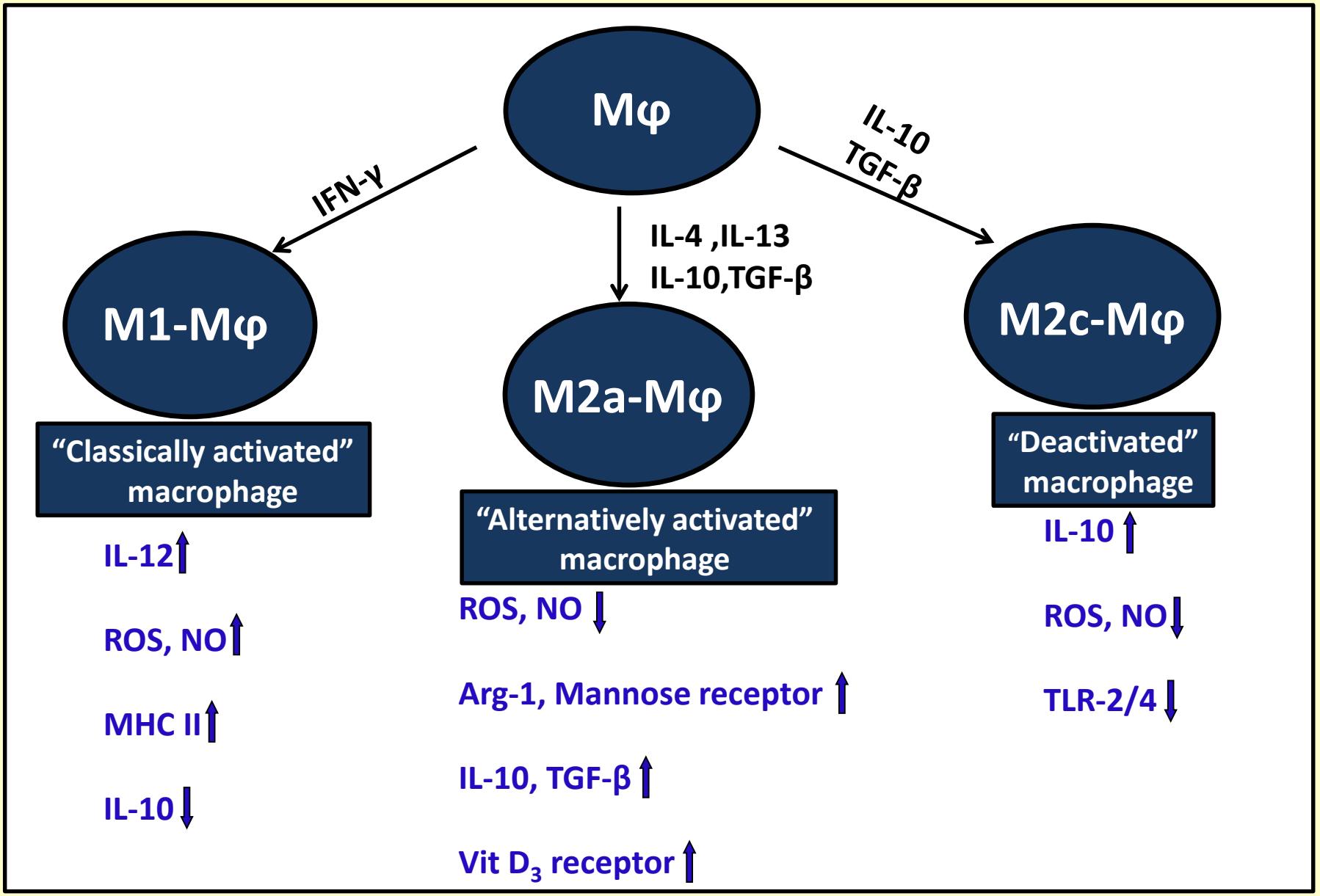






Mukhopadhyay et al., 2015 PLoS Negl Trop Dis

Activation status of monocytes/ macrophages



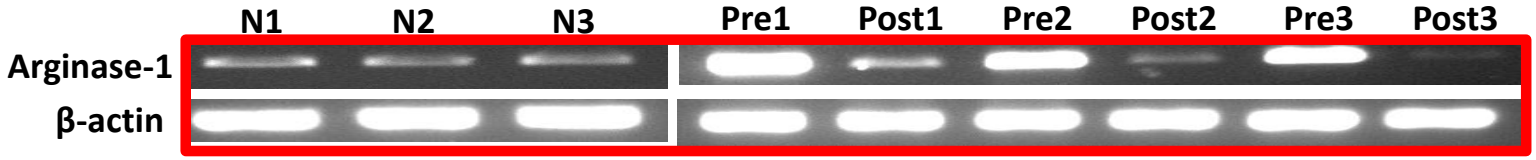
Status of circulating cytokines in PKDL

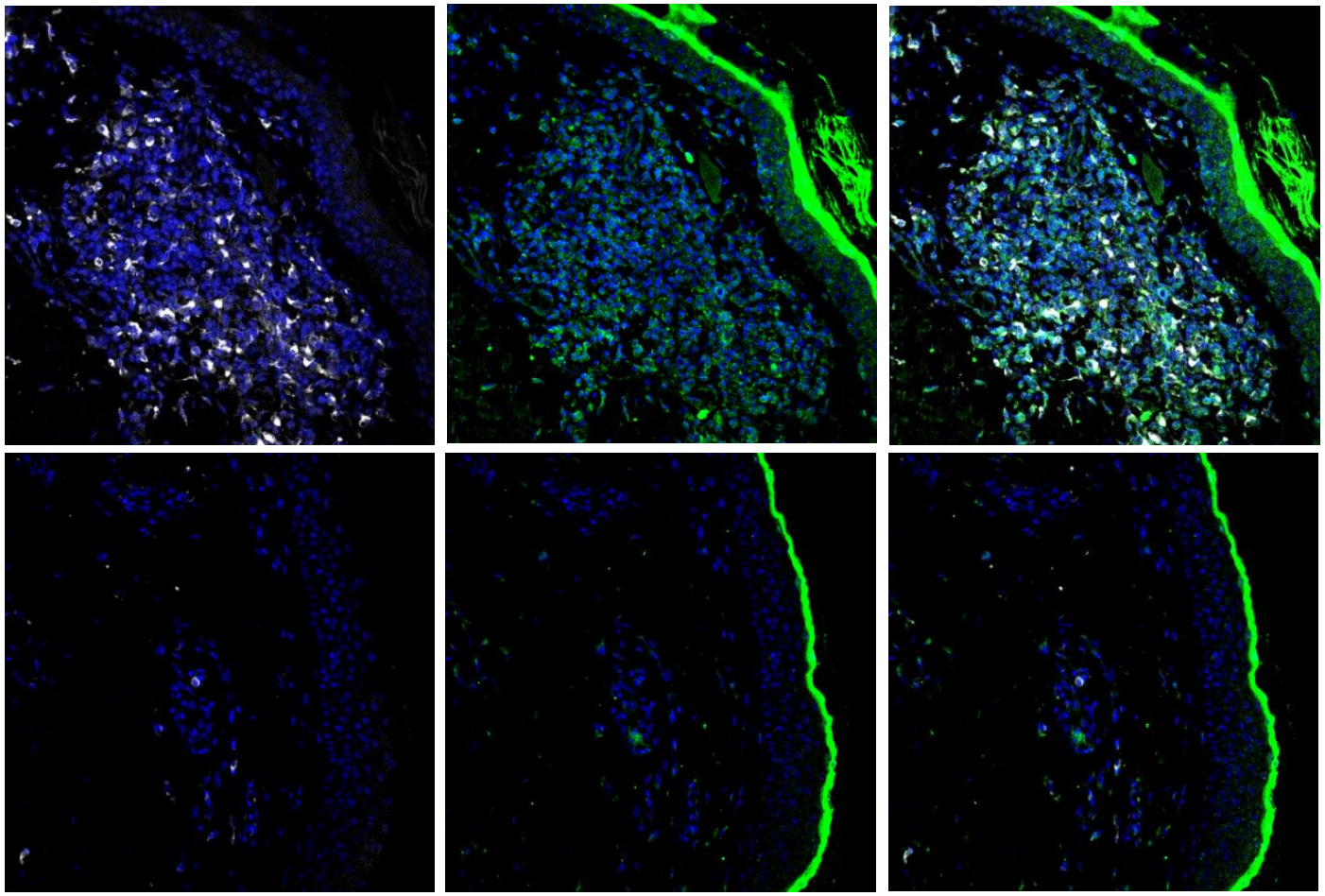
Cytokines (pg/ml)	Healthy controls (n =15)	Pre treatment (n = 35)	Post treatment (n =30)
IL-6	53.94 ± 29.29	63.10 ± 57.78	255.60± 299.40$^{@}$
IL-1β	7.20 ± 9.08	7.44± 10.69	89.81 ± 131.70$^{@@}$
TNF-α	6.70 ± 7.72	81.43 ± 77.75***	346.00 ± 495.90***
IL-8	82.98 ± 111.80	938.00 ± 1264.00*	2271.00 ± 2060.00$^{***,@}$
IL-10	12.68 ± 6.50	33.60 ± 28.84*	21.33 ± 18.64$^{@}$
IL-4	61.35 ± 20.12	133.30 ± 70.48**	107.50 ± 52.24
IL-13	35.13 ± 21.68	184.10 ± 235.00	83.21 ± 136.10
TGF-β	5980.00 ± 4061.00	17953.00 ± 17704.00*	11532.00 ± 6209.00

Data are mean ± S.D. $^{@}p<0.05$, $^{@@}p<0.01$, $^{@@@}p<0.001$, significantly different than presentation; $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ significantly different than healthy controls. Kruskal Wallis test followed by Dunn's multiple comparison test was performed.

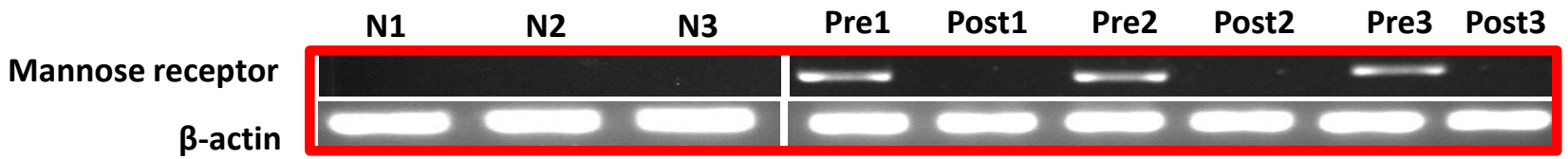
Mukhopadhyay D. *et al. J Inf Dis* 2011; 204(9):1427-36

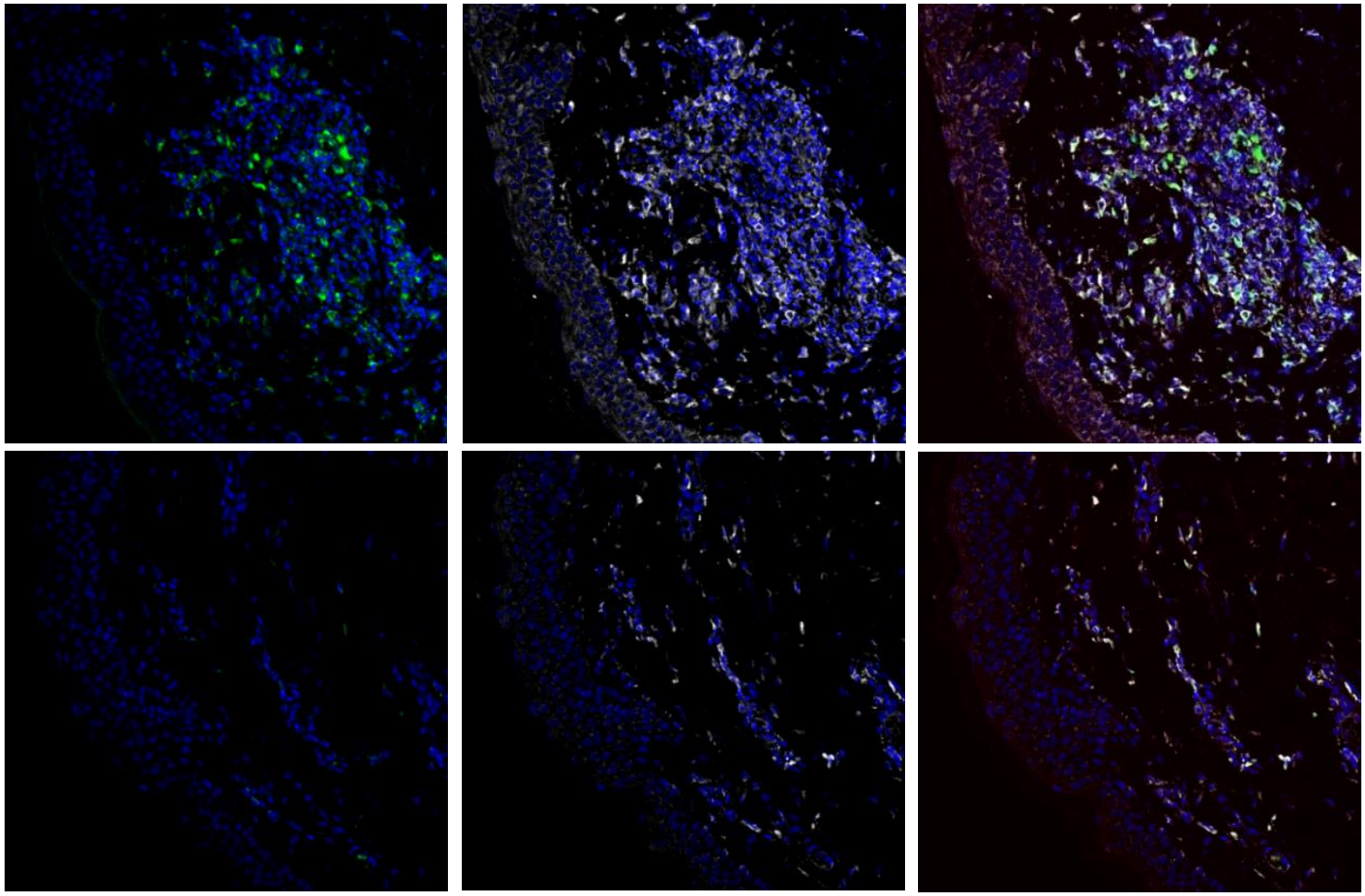
Enhanced expression of Arginase-1 in dermal macrophages



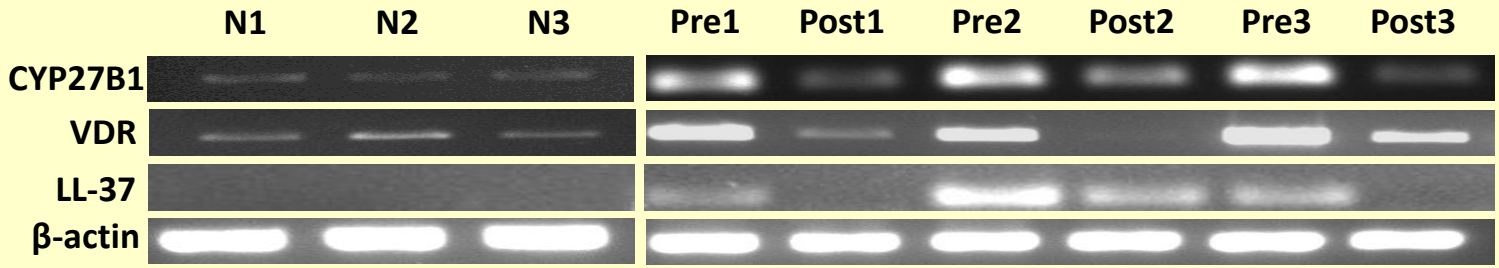


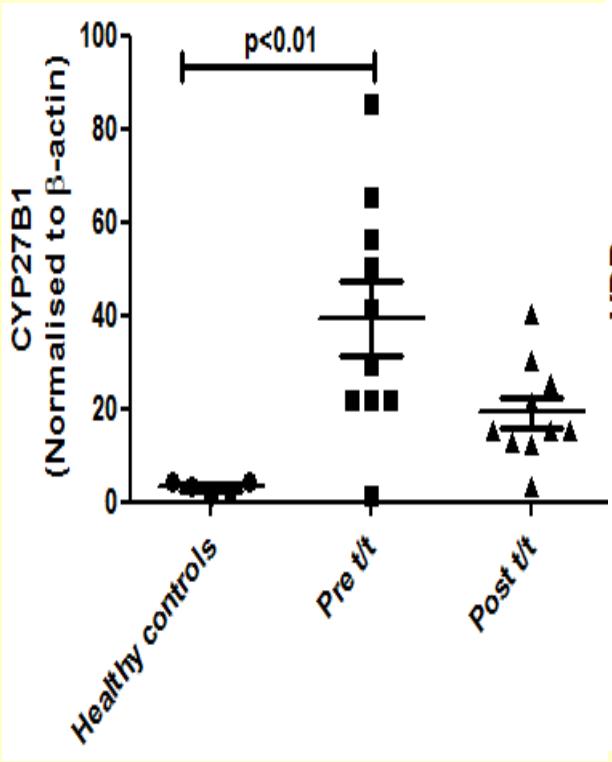
Raised expression of mannose receptor in dermal macrophages

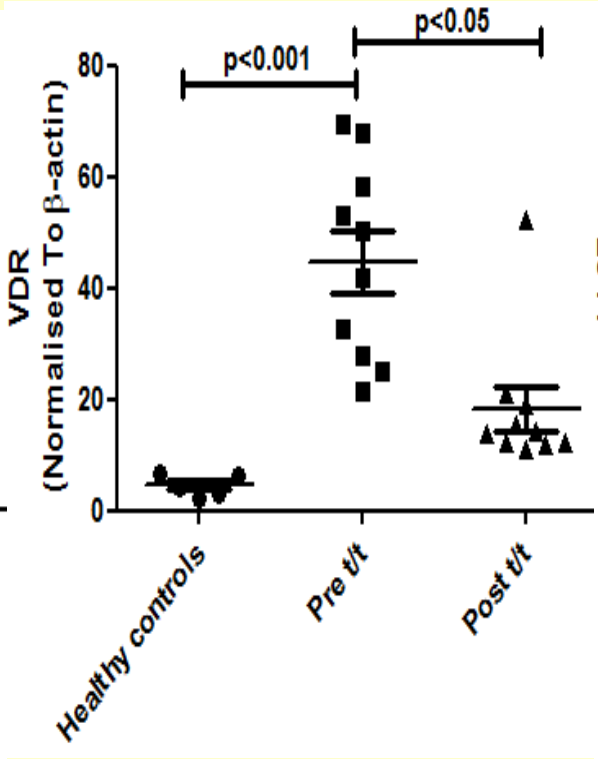


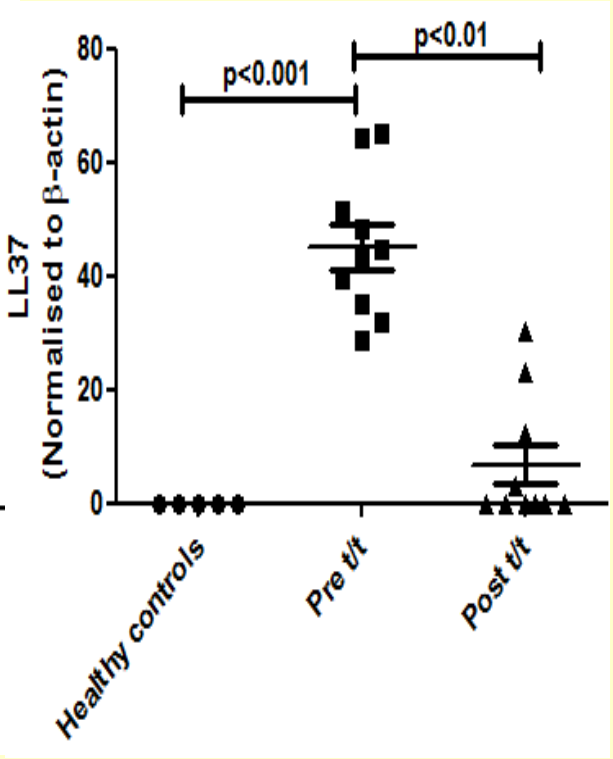


Lesional expression of Vit-D_3 signaling associated genes









Taken together…

- **Active surveillance, detection and effective treatment of PKDL is critical for achieving elimination of Leishmaniasis in South Asia.**

- **Parasites generate an immunosuppressive milieu to ensure their survival.**

- **Drugs with parasiticidal and immunomodulatory properties e.g. Miltefosine are more likely to ensure sustained parasite clearance.**

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