Conclusion: Together, our findings indicate dynamic changes to macrophage and monocytes populations in VL patients over the course of drug treatment, and suggest that the functions of these cells may change at different stages of disease. We found upregulation of some markers for monocyte deactivation.

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Microplate whole blood interferon-γ release assay for marker of Leishmania donovani infection

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Background: Laboratory tests which can be produced in a reproducible and scalable manner are much needed to identify visceral leishmaniasis (VL) infection and fulfill the goals of the elimination campaign. Whole blood interferon gamma (IFN-γ) release assay (IGRAs) is an *in vitro* immune test that has recently been developed as an alternative to the Leishmanin skin test (LST) for identification of individuals exposed to *L.donovani* infection but without disease. Requirement of 3 ml of blood preclude this test widely acceptable for larger community based studies. This study aimed at evaluating the performance of microplate based IGRA using 300 μl blood (direct blood as well as 1:1RPMI diluted blood) with conventional IGRA (3ml blood) to establish this assay as an epidemiological tool for marker of infection.

Methods & Materials: We employed conventional IGRA and microplate based IGRA with direct as well as diluted venous blood using soluble leishmania antigen (SLA) on patients with active visceral leishmaniasis (VL, n = 32), patients with cured VL (n = 20), endemic Healthy Controls (EHC, n = 21) and healthy control subjects living in non-endemic area (NEHC, n = 12). IFNγ levels in culture supernatants were measured by ELISA and kappa statistics was used to access the concordance between test assay formats.

Results: The whole blood cells of both active VL and cured VL produced significantly level of IFN-γ in both format of IGRA. Positive correlations were found with active VL blood in IFN-γ production between 3 ml vs 600 μl, 3 ml vs 300 μl, and 600 μl vs 300 μl, while with cured VL blood it was moderate with 3 ml vs 600 μl; 3 ml vs 300 μl. No significant difference in the overall IFN-γ response by both assay formats was detected, and agreement between tests was significant.

Conclusion: We demonstrate a reliable and scalable process similar in sensitivity to conventional IGRA, but with the advantage of 10 times less venous blood requirement and higher through-put. Development of microplate based IGRA format will be useful tool for providing the means to more efficient screening in large scale immunological and epidemiological studies and fill an unmet need in the VL elimination campaign on the Indian sub-continent.

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Decreased miltefosine susceptibility in clinical isolates of Leishmania donovani derived from visceral leishmaniasis and post kala-azar dermal leishmaniasis: Apparent mechanisms and clinical implications

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Background: Miltefosine is an oral antileishmanial drug. Recent reports indicate a significant decline in its efficacy and high relapse rate in visceral leishmaniasis (VL) and post kala-azar dermal leishmaniasis (PKDL). Miltefosine susceptibility of relapse case isolates were >3 fold lower compared to pre treatment isolates.

Methods & Materials: To understand mechanism responsible for miltefosine unresponsiveness, we determined (i) the sequence of LdMT and LdRos genes implicated in miltefosine translocation (ii) miltefosine accumulation and reactive oxygen species (ROS) tolerance and (iii) transcriptome profile in clinical isolates of Leishmania donovani obtained from VL and PKDL patient at pre treatment and clinically relapsed stages as well as miltefosine resistant parasite(LdM30).

Results: LdMT gene sequencing revealed the previously reported single-nucleotide polymorphism, C1259→A resulting in substitution of Thr 420→Asn and a novel SNP T527-A resulting in substitution of Val176-Asp resistant parasites. L. donovani parasites from VL and PKDL patients relapsed after miltefosine treatment exhibited significantly lower accumulation of miltefosine compared with wild type parasites. Miltefosine induced ROS levels were significantly low ($p<0.05$) in macrophages infected with LdM30 and parasites from relapse cases compared to wild type parasites, indicating better tolerance for oxidative stress in unresponsive clinical isolates. Transcriptome profiling revealed that several genes involved in antioxidant defense mechanism (TRYP, Cyt b5 Red, TSH), metabolic process (Lipase precursor, PGMPUT), transporters (VPTM, MDRP), cell component and cell motility (SMP2, NUP155, CYP) are preferentially expressed in LdM30 and relapsed case parasites than wild type L. donovani parasites. Several other genes mainly transporters like ABCF2, amino acid transporter, surface acylated putative protein, APH and mitochondrial precursor peptide, chaperon TCP20, clathrin coated assembly protein, C5 sterol desaturase, autophagy protein ATG10 were preferentially expressed in wild type parasite compared to relapse case and LdM30 parasites.

Conclusion: The present study provides the understanding of parasitic factors and pathways responsible for miltefosine unresponsiveness in VL and PKDL. Decreased miltefosine susceptibility and increasing relapse rate in VL and PKDL patients indicate the declining efficacy of monotherapy with miltefosine and warrants the need of introducing alternate drugs/ combination therapy with miltefosine.

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