PhD defence Keshav Rai

Development of tools to determine the phenotype and genotype of Leishmania donovani for tracking treatment failure in anthroponotic visceral leishmaniasis in Nepal.

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Dit is de omschrijving

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Abstract

Leishmania (Leishmania) donovani is a protozoan parasite that causes visceral leishmaniasis (VL) in the Indian subcontinent (ISC) and the parasite is transmitted between different human hosts by the bite of sand flies. For more than half a century, pentavalent antimony (SSG) was the drug applied in the treatment of VL but later on the emergence of SSG-resistant parasites in ISC affected its efficacy. Hence, miltefosine (MIL) has been used as a first line drug in VL treatment between 2004 and 2012 in order to improve the VL treatment in ISC. The Kala-azar Elimination Programme (KAEP), a collaboration programme between the governments of Bangladesh, India and Nepal which aims to reduce the VL infection down to 1 in 10,000 population at upzilla, sub-district and district levels of respective countries. However, MIL failure are reported in ISC, the mechanism of miltefosine treatment failure is not completely understood. We hypothesize that several parasite factors have significant contribution in the treatment failure of VL infection in Nepal. Therefore, this study aimed at assessing parasite factors and evaluating whether the parasite has a significant role in the treatment efficacy of available drugs such as SSG and MIL. First, we aimed to characterise the drug resistant phenotype of clinical parasite isolates from Nepalese VL patients. The increased rate of MIL treatment failure (20%) was observed shortly following the introduction of MIL as a first line therapy. Hence, the parasites were isolated from patients recruited in the B.P. Koirala Institute of Health Sciences (BPKIHS), Dharan and patients were followed up till one year in order to find the treatment outcome. Later on, we correlated the in vitro parasite phenotype to clinical treatment outcome. The promastigote MIL susceptibility (inhibitory concentration 50%, IC50) of clinical isolates from MIL cure was not significantly different from MIL relapse. Although reduced susceptibility was observed in a few strains, natural parasite resistance is thus not likely involved in MIL treatment failure. Indeed, the parasite MIL susceptibility assay did not appear to be an efficient predictor of MIL treatment failure. It seems that the increasing treatment failure was not driven by drug exposure. Although the currently applied tools for the epidemiological study of treatment failure are based on in vitro biological assays for testing drug susceptibility of the parasite, these assays seem inadequate to assess parasite factors in relation to MIL treatment failure. Therefore, parasite phenotypes other than drug resistance such as virulence, infectivity are urgently required to explore in order to understand the corresponding in vivo treatment outcome that can influence the VL epidemiology and transmission in ISC. To approach other phenotypes of the parasite that can affect treatment efficacy, this study evaluates the parasite virulence by assessment of the parasites' capacity for metacyclogenesis and macrophage infection. In fact, parasite metacyclogenesis determines infective capacity and reflects the fitness to infect the host. Interestingly, we found a significant association between the number of metacyclic parasites, parasite infectivity, and patient treatment outcome in the Indian subcontinent. Together with previous studies on resistance of L. donovani against pentavalent antimonials, these data suggest that the infectivity of the parasite, or related phenotypes, might be a more determinant factor for treatment failure in visceral leishmaniasis than drug susceptibility. The laboratory approach thus showed that MIL failure parasites have increased infection and in vivo survival skills than the MIL cure parasites. Hence, current evidence shows that the parasites' infectivity in relation to VL treatment failure must be considered, and this is not detected by drug susceptibility assays. Genetically homogeneous populations of Leishmania donovani parasites cause VL in Bangladesh, India, and Nepal.

Classical approach to determine protozoa diversity in other regions of the world, such as microsatellite typing, have proven of little use in the area, as they are not able to discriminate circulating genotypes. In fact, recent whole genome sequencing (WGS) already identified 10 different populations named as ISC001-ISC010. On top of WGS analysis, we developed the Single Locus Genotyping (SLG) assay that identifies apomorphic single point mutation (SNP) of the seven genotypes in the ISC whereas such assay could not be designed for three composite genotypes. This assay was optimized and applied on parasite isolates collected in Nepal between 2011 and 2014. Together with WGS data, 204 strains collected in the period 2002-2011, we provide a proof-of-principle for the application of genotyping to track the parasite populations in differential geographic distribution. Another objective of this study was to assess candidate genetic markers in parasites that could have close relation with the treatment failure. Even though an antimony resistant genotype was identified that correlated with clinical outcome, none of the genotypes were found to correlate with miltefosine treatment failure. These facts highlight the potential application of SLG assay for epidemiological follow-up of VL transmission in the post-elimination phase in the ISC. Finally, the overall finding of this study demonstrates that a multi-factorial approach to determine parasite characteristics is needed to study treatment failure. In future, this approach would also enable to pave the way to track the reduced efficacy of treatment against the VL disease. However, more improvements in technologies to assess the phenotype and genotype of parasites are required that can boost the VL elimination programme by understanding the molecular epidemiology and transmission dynamics in the ISC.