Short Report

Genomic DNA extraction and amplification of *Leishmania donovani* using polymerase chain reaction (PCR) from archived, Giemsa- stained slides

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Abstract

In Sri Lanka, diagnosis of cutaneous leishmaniasis **(**CL) is usually based on clinical features and direct microscopy examinations. Polymerase chain reaction (PCR) for *Leishmania* is usually performed on tissue samples. In this study, we extracted DNA from archived Giemsa-stained slides which were prepared from cutaneous lesions. A total number of 85 Giemsa-stained slides fixed between 2008-2017 were selected. All the slides were examined using light microscopy and the number of amastigotes in positive smears was recorded. A nested PCR was carried out to amplify the 385 bp fragment of *L. donovani* kinetoplast mini-circle sequence. All 40 positive slides had only 1-2 amastigotes per slide. Of these, only 20% were PCR positive. Of the 45 negative slides, only one gave positive PCR result. Further studies are required to confirm the efficacy of PCR on Giemsa-stained smears in our setting.

Keywords: *Cutaneous leishmaniasis, Giemsa-stained smears, Leishmania donovani, Sri Lanka*

Introduction

Leishmaniasis is a vector borne disease caused by *Leishmania* species. A phlebotomine sandfly species, mainly *Phlebotomus argentipes* acts as the vector.[1] Leishmaniasis is an endemic disease in Sri Lanka.[2] There are previous reports from Iran and Nepal that used PCR on archived Giemsa-stained slides to identify the species for the diagnosis of CL and visceral leishmaniasis (VL).[3-6] This method has been used to identify other leishmania species; *L. infantum* and *L. major*.[3] In Sri Lanka, this is the first attempt to assess the possibility of using PCR for identification of *L. donovani* using DNA extracted from archived Giemsa-stained slit skin smears.

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Methods

Stored Giemsa-stained slides at the Department of Parasitology, Faculty of Medicine, University of Peradeniya, Sri Lanka were used for this study. Each sample was taken from skin lesions on suspected cases of CL. Slides were fixed between 2008-2017. Most of the slides except for the ones from recent years were in poor condition, and some were covered with dust due to oil residues from the oil immersion lens. Of the 85 slides used, 40 were previously determined as positive by direct microscopy examination and 45 slides as negative. All the slides were re-examined with a light microscope (Carl Zeiss™ PrimoStar™, Germany) under high magnification (1000x). Positive slides were confirmed and the numbers of amastigotes in positive slides were recorded. All negative slides were reconfirmed as negative.

Giemsa-stained smears were wiped with tissue paper to remove any oil used in the microscopic examination. The dry smear was scraped from its slide using a sterile scalpel. Genomic DNA extraction was carried out using a commercial DNA extraction kit (Invitrogen™PureLink™ Genomic DNA Mini Kit, Thermo Fisher Scientific) following the manufacturer's protocol. Extracted DNA from each sample was stored at -20 °C. The nested PCR amplification was carried out for a fragment of *L. donovani* species specific kinetoplast mini-circle sequence. Primers and PCR conditions were used as given in a previous study.[7] The amplification was performed in a thermal cycler (NYX TECHNIK). PCR products were subjected to 1.5% agarose gel electrophoresis. The gel was dyed with Diamond™ Nucleic Acid Dye (Promega Corporation, USA) and observed under UV light.

Results

All the 40 Giemsa stained positive slides had only 1-2 amastigote counts per slide. Of these slides, only 8 (20%) were PCR positive. Of the 45 negative slides, only one gave a positive PCR result. All the slides that gave positive PCR results were collected within the past one year. Intense bands at 385 bp were observed for PCR positive samples.[7]

Discussion

In this study we used archived Giemsa stained slides to identify *L. donovani* parasites at species level using PCR. In a study conducted by Kazemi-Rad et al. (2008),[3] a statistically significant difference was observed between microscopy and PCR,[3] with PCR positivity of 89.6% for the smear positive slides. A study conducted by Motazedian et al. (2002)[2] showed high PCR positivity for smear positive slides with more than 50 amastigotes per slide. However, they reported less sensitivity in slides with 1-20 amastigotes.[2] In the present study, all positive slides used in this study had only 1-2 amastigotes and of these, only 20% were PCR positive. The high percentage (80%) of negative results may have been caused by the poor state of slide conservation.[3] Also, it is possible that the very few amastigotes on the slide preparation were not included in the scrapings taken for the PCR

A previous study showed the possibility of extraction and PCR amplification of *Leishmania* DNA from cutaneous skin smear slides stored up to 36 years.[8] This study reported a high percentage (29.8%) of negative results with slides from the first 11 year period compared to the more recently prepared slides.[8] Studies that used CL skin smear slides prepared more recently have reported contrasting results with a higher PCR detection rate for the positive

slides.[2] In the present study, all the slides that gave positive PCR results were collected within the previous one year.

Limitations of the present study and future directions

Careful slide storage and preservation would increase the sensitivity of this method. Archiving another set of Giemsa-stained slides instead of the slides observed under oil immersion lens would increase the chance of extracting DNA and would improve the condition of the stored slides. This study needs to be extended using slides with different amastigote counts, showing a wide range. Examination of the slides again after collecting the scrapings, to make sure that there are no amastigotes left on the slide may also be helpful. The use of PCR to detect leishmanial DNA in Giemsa-stained smear scrapings can be used in investigations of the reservoir hosts and in research conducted with infected animal models[9] and therefore, this technique can be applied in Sri Lanka to identify the reservoir hosts. A previous study conducted by Beldi et al., (2017)[10] has confirmed the usefulness of slides for PCR identification of *Leishmania* parasites in retrospective epidemiological investigations.[10] Therefore, this method can be adopted for similar studies conducted in Sri Lanka.

Conclusions

In the present study, all the slides that gave positive PCR results were one year old or less. Further studies are required to confirm the usefulness of PCR on Giemsa-stained smears in our setting. Careful slide storage and preservation could increase the sensitivity of this method.

Conflict of interests

The authors declare that there is no conflict of interest regarding the publication of this article.

Author declaration:

AA conducted the laboratory work and manuscript writing. DI conducted the manuscript writing and editing

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