论著

内脏利什曼病患者骨髓及血内病原体特异性kDNA片段PCR扩增用于诊断的研究

胡孝素1,杨文天1,马莹1,陈建平1,何生全2,许庭玺3,谢孝全3

- 1 华西医科大学寄生虫学研究室 成都610041
- 2 四川省南坪县人民医院内儿科 南坪623400
- 3 四川省汶川具人民医院 汶川623000

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摘要

目的:建立简易、准确的诊断内脏利什曼病和病原体鉴定技术。方法:采用作者设计的杜氏利什曼原虫 (L. d.) 种特异引物I和II, 经PCR 扩增样品内病原体kDNA 297 bp 片段,检测22 例确诊内脏利什曼病 (VL) 患者骨髓、血、血清共55 份样品和4 例临床疑诊为黑热病患者的骨髓(共26 例,59 份样品)。结果:(1) PCR 法与骨髓涂片镜检符合率为96.2%(25/26);(2) PCR 法检测骨髓、血及血清的总阳性率为95.4%(21/22);(3) PCR 法检测22 例骨髓、16 例血样品及17 例血清样品,阳性率分别为91%(20/22)、68.8%(11/16)及29.4%(5/17)。15 例骨髓对照样品得自9 例白血病患者及6 例健康人。血及血清对照样品各得自5 例健康人。全部对照未见扩增产物,均为阴性。结论:采用引物I和II进行PCR扩增检测血内kDNA 特异片段诊断内脏利什曼病有较好前景。

关键词 内脏利什曼病 诊断 PCR kDNA 骨髓 血 血清

分类号

STUDIES ON DIAGNOSIS OF VISCERAL LEISHMANIASIS BASED ON PCR AMPLIFICATION OF *LEISHMANIA DONOVANI* kDNA FRAGMENT FROM BONE MARROW AND BLOOD SAMPLES OF VISCERAL LEISHMANIASIS PATIENTS

Hu Xiaosu1, Yang Wentian1, Ma Ying1, Chen Jianping1, He Shengquan2, Xu Tingxi3, Xie Xiaoguan3

- $1 \quad Laboratory \ of \ Parasitology \ , \ West \ China \ University \ of \ Medical \ Sciences, \ Chengdu \ 610041$
- 2 Nanping County People's Hospital, Nanping 623400
- 3 Wenchuan County People's Hospital, Wenchuan 623000

Abstract

AIM: To develop a simple and accurate technique for the diagnosis of visceral leishmaniasis (VL) and identification of Leishmania pathogen. METHODS: Based on a set of oligonucleotide primers I and II designed by the authors with L. donovani specificity, a polymerase chain react ion (PCR) was conducted to amplify a minicircle kDNA fragment (297 bp) for identification and detection of L. donovani in the bone marrow, blood and serum samples from 22 confirmed VL patients and 4 bone marrow samples from 4 clinically diagnosed cases (59 samples collected from 26 cases). RESULTS: (1) The coincidence rate between PCR and bone marrow smear method was 96.2% (25/26); (2) The total positivity of PCR with the bone marrow, blood and serum was 95.4% (21/22); (3) The positive rate of PCR amplification with bone marrow, blood and serum specimens were 91.0% (20/22), 68.8% (11/16) and 29.4% (5/17), respectively. The controls included 15 bone marrow samples from 9 leukemia patients and 6 normal persons; 5 blood and 5 serum samples of normal persons. No amplification product was showed from all of the controls. CONCLUSION: The result shows that the diagnosis of visceral leishmaniasis based on detecting kDNA in blood by PCR amplification with primers I and II is promising.

Key words <u>Visceral leishmaniasis</u> <u>diagnosis</u> <u>PCR</u> <u>kDNA</u> <u>bone marrow</u> <u>blood</u> serum

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