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DIAGNOSIS OF CUTANEOUS LEISHMANIASIS USING RESTRICTION ANALYSIS OF KINETOPLAST DNA POLYMERASE CHAIN REACTION PRODUCTS

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Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Biochemistry at Al-Quds University-Palestine

September 2001

ENDORSEMENT

Title of thesis:

Diagnosis of Cutaneous Leishmaniasis Using Restriction Analysis of kinetoplast Minicircles DNA-Polymerase Chain Reaction Products

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ACKNOWLEDGMENTS

I would like to thank my supervisor Dr. Haroon Khanfar, and Dr. Ziad Abdeen for enabling me to carry out this research and help.

I would like to express my deep and sincere appreciation to Dr. Carol L. Eisenberger for her encouragement, advice and I admire her wisdom open mind and kindness.

My sincere appreciation and grateful to Dr. Abed Alkareem Alsherif for constant support.

I am indebted to my dear brother Mohammed and my dear friends Miss. Obaida Ali Yousef and Ghadeer Za'tarah for their encouragement and help through out this study.

I would also like to thank Dr. Khaled Qabaha, Mr. Abed Elmajeed Naseraldeen; Mr. Samir Sawalha; Mr. Omar Hamarsheh; and Dr. Gerlind Anders for help and collecting samples throughout this study.

Thanks are also extended to the staff of Arab Health Center in Jerusalem headed by Dr Adnan Arafah and Mr. Ahmad Soror for their constant support.

I would like to thank the Deusche Forschungs Gemeinschaft. DFG, for financial support.

List of Abbreviations

A	Arava
Bp	Base pair
BPB	Bromo Phenol Blue
BSA	Bovine Serum Albumin
CL	Cutaneous Leishmaniasis
DCL	Diffuse Cutaneous Leishmaniasis
DMSO	Di Methyl Sulf Oxide
EF	Excreted Factor
EG	Egypt
ELISA	Enzyme-Linked Immunosorbent Assay
ET	Ethiopia
FCS	Fetal Calf Serum
HIV	Human Immunodifeciency Virus
IL	Israel
IN	India
IQ	Iraq
ITS1	Internal Transcribed Spacer
J	Jericho
JO	Jordan
J.V	Jordan Valley
K.A	Kfar Adumim
KDNA	kinetoplast DNA
KE	Kenya
Laet	Leishmania aethiopica
Li	Leishmania infantum
Lm	Leishmania major
LR	Leishmaniasis Recidivans
LRC	Leishmania Reference Center
Lt	Leishmania tropica
MCL	Muco Cutaneous Leishmaniasis
MW	Molecular Weight
N	Negev
OD	Optical Density
PBS	Phosphate Buffered Saline
RFLP	Restriction Fragment Length Polymorphism
PCR	Polymerase Chain Reaction
Ph. Papatasi	Phlebotomus Papatasi
PPIP-PCR	Permissively Primed Intergenic Polymorphic Polymerase
Chain Reaction	
P. Obesus	Psammomys Obesus
PS	Palestine
Rpm	revolutions per minute
SL	Spliced Leader
SD	Sudan
SN	Sanegal
SU	Soviet Union
SSU rRNA	Small Subunit Ribosomal RNA
T	Tiberias
TN	Tunisia

TR	Turkish
U	Uvda
UV	Ultra Violet
UZ	Uzbekistan
VL	Visceral Leishmaniasis
W.B	West Bank
WHO	World Health Organization

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ABSTRACT

Cutaneous leishmaniasis is generally characterized by skin lesions. Localized lesions that heal spontaneously over a few months are usually caused by *L. major*, with *Psammomys obesus* and *Meriones crassus* as the most important reservoir hosts. Lesions that may last up to several years are usually caused by *L. tropica.* Identification and characterization of *Leishmania* parasites from patients, as well as from reservoir hosts, is important for understanding the epidemiology of leishmanaisis. In addition, fast and accurate diagnosis is important for providing the correct patient care.

We have employed a kinetoplast DNA (kDNA) based polymerase chain reaction (PCR) technique for direct diagnosis of *Leishmania* parasites from tissue samples. The PCR reaction utilizes the Uni21/Lmj4 primer pair, originally designed to amplify the full *L. major* minicircle DNA. In fact, this primer pair can amplify all Old World *Leishmania* minicircles including *L. tropica, L. aethiopica,* and the *L. donovani complex*. As minicircle of the different species have similar sizes, ranging between 650 - 850 bp, we applied restriction endonucleases (MboI, RsaI, BanII, HpaII, HaeIII). We optimized the method so that restriction enzyme analysis of kinetoplast DNA can aid in diagnosis and species identification. In this project, results show that RsaI, MboI and HaeIII give different restriction patterns for all species and can discriminate between distant regional isolates of both *L. major* and *L. tropica.* Both standard and high fidelity Taq polymerases were used for amplifying the kDNA minicircle of leishmanial strains. Using the standard Taq polymerase, we obtained different restriction patterns for each Old World