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Study Of Comparison Between Traditional Methods And Molecular Diagnosis Of Cutaneous Leishmaniasis In Al- Muthanna Province

A Thesis

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Abstract

This study aimed to compared the sensitivities and specificities of PCR assays used for parasite identification with leishmanial culture and microscopic detection in order to validate these PCR techniques for the molecular diagnosis of cutaneous leishmaniasis .

Mean age of patients' group was 14.4±11.94 years with a range of (1-45 years) while the mean age of control group was 13.75±11.81 and a range of (2-46 years) . Regarding gender , patients group included 45 males (72.58%) and 17 females (27.42%) suggesting that the disease is more common in males with a male to female . Mean age of male patiens was 16.1+12.92 years , while mean age of female patients was 9.91+7.42 years .

The distribution of patients according to residency was as follows : 23 patients (37.15 %) from Al-Warkaa , 10 patients (16.13 %) from Al-Hillal , 9 patients (14.52 %) from Al-Khther , 8 patients (12.9 %) from Al-Salman , 4 patients (6.45 %) from Al-Mamlaha , 3 patients (4.84 %) from Center , 2 patients (3.23 %) from Al-Swir, one patient from Al-Majd (1.61%) , one patient from Al-Najmi (1.61%), one patient from Al-Draji (1.61%) .

Three methods were used to identify the presence of the parasite for purpose of comparison . These were Light Microscopic examination , culture and PCR .

The *Leishmania subtypes in* the present study were distributed as follows: *L. tropica* accounted for 69.35% while *L. major* accounted for 22.58% of cases .

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List of Abbreviations

A	Adenine
APS	Ammonium peroxodisulfate
ARF	Annual Rainfall
bp	Base pair
BLAST	Basic Local Alignment SearchTool
BM	Bone marrow
BSA	Bovine serum albumin
C	Cytosine
CA	Central Asia
CE	Capillary Electrophoriesis
CL	Cutaneous leishmaniasis
DAT	Direct agglutination test
DCL	Diffuse cutaneous leishmaniasis
ddH2O	Double distilled water
Dμ2 (δμ2)	Ddm Delta mu squared
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
DMSO	Dimethyl-sulfoxid
Dps	Proportion of shared alleles
EDTA	Ethylenediamine-tetra acetic acid

fg	Femtogram
FP	Filter papers
Fis	In-breeding coefficient
Fst	F-statistic
G	Guanine
HCl	Hydrochloric acid
He	Expected heterozygosity
Ho	Observed heterozygosity
IMM	Infinite Allele Model
ITS	Internal transcribed spacer
K	Number of population
KZ	Khazakistan
Kbp	Kilo base-pair
KDNA	Kinetoplast DNA
MAJ	*L. major*
ME	Middle East indicating South West Aisa
MCL	Mucocutaneous leishmaniasis
MCMC	Markov chain Monte Carlo
MLEE	Multilocus enzyme electrophoresis
mRNA	Messenger RNA
mtDNA	Mitochondrial DNA

NNN	Novy-MacNeal-Nicolle medium
NW	New World
NJ	Neighbor-joining tree
NWA	North West Africa
OIF	Oil immersion field
OW	Old World
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PAGE	Polyacrylamide gel electrophoresis
PKDL	Post kala-azar dermal leishmaniasis
Pg	Picogram
PS	Palestine
RFLP	Ristriction fragment length polymorphism
RNA	Ribonucleic acid
RR	Relative risk
rRNA	Ribosomal RNA
RT-PCR	Reverse-transcriptase PCR
SDS	Sodium dodecyl sulphate
SMM	Stepwise Mutation Model
sp.	Species
ssU RNA	Small sub-unit RNA

T	Thymine
Taq	*Thermus aquaticus*
TBE	Tris borate EDTA
TE	Tris EDTA
TEMED NNNN-	Tetramethylene diamine
TM	Turkmenistan
U	Unit
U	Uracil
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
UV	Ultra violet
UZ	Usbekistan
VL	Visceral leishmaniasis
WHO	World Health Organization

1.1- Introduction

Leishmaniasis are infection of parasites attributable to a range of *Leishmania* parasites supported by a broad variety of vectors and reservoirs spread on all occupied continents and caused by more than 20 species of *Leishmania* and a in nature common severe infection , it includes cutaneous leishmaniasis , visceral leishmaniasis and mucocutaneous leishmaniasis (Herwaldt , 1999) . Adler and Theodor, (1957) showed that 350 million citizens are at hazard in 88 countries ,(66-22) of which are in the Old World and the New World respectively , and 72 in the emergent countries , with generally rate of (1–1.5) million cases of cutaneous leishmaniasis and 500,000 cases of visceral leishmaniasis .

Cutaneous leishmaniasis is attributable to *Leishmania braziliensis* complex and *Leishmania mexicana* in the New World and by *Leishmania tropica* , *Leishmania major* and *Leishmania aetiopica* complex in the Old World (Ashford ,1996 and 1999 and 2000) . It has been expected that 350 million people are at danger with 500,000 new visceral leishmaniasis cases each year , definite cases of visceral leishmaniasis have been reported from 66 countries , 90% of the world's visceral leishmaniasis load occurs on the East Africa (Sudan , Ethiopia and Kenya) , Indian sub continent (India , Bangladesh and Nepal) and Brazil (WHO1991, 1996 and 1998) . About 90% of cases occur in Iraq , Iran , Syria , Saudi Arabia , Algeria , Afghanistan , Peru , and Brazil (Al-Jawabreh *et al*., 2003).

Cutaneous leishmaniasis is a polymorphic disease , its symptoms ranging from asymptomatic infection to mild nature - limited cutaneous ulcer or to more delayed and common lesions (Al-Majali *et al*., 1997) . This scientific polymorphism may result from changeability either in the parasite pathogenic variety or in the host immune response . This heterogeneity is reflection to result in regular accumulation of different

mutations , sexual recombination , genetic replace and hybridization (Arda and Kamal , 1983) .

Finding of Cutaneous leishmaniasis is hazardous because of the high cost and major toxicity of recent treatment regimens (Vega-Lopez , 2003) . As well , for in cooperation epidemiological and clinical reasons , it is main to recognize the *Leishmania* species in each area , even though different species need various administrative methods , different *Leishmania species* can cause also appearing cutaneous lesions in the like ecological region , unfortunately, the traditional investigative techniques for Cutaneous leishmaniasis have some limits (Al-Rai , 2005) .

Microscopic examinations are inexpensive and rapid , but they have short compassion mostly in chronic lesions (Al-Rai , 2005) . At the same time as , cultures of *Leishmania* are more sensitive , they are vulnerable to microbiological corruption and difficult attributable to exacting growth supplies of special strains (Anis *et al*., 2001) . Also , some strains grow fine over than others *in vitro* and this detail causes careful growth of essential strains through culture in varied infections (Ashford , 1999) .

Molecular techniques , for example polymerase chain reaction (PCR) , permit strict recognition and description of parasites in isolates obtained from patients (Barker, 1989) . Molecular techniques designed for species resolve , PCR-RFLP is proved to be the most susceptible and specific practice (Ayala, 1998) . Skin biopsy and as well materials obtained by skin cut/exudates have been used for PCR in different studies (Ben-Ismail *et al*., 1997) .

1.2 Aims of the study :

The aim of this study was to isolate and identify the cutanoeus leishmaniasis and compare several methods of diagnosis of parasites .