Molecular Diagnosis and Genotyping of *Entamoeba spp* Among Infected Egyptians

A Thesis

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By

Mohamed Hasan Mohamed Roshdy
(B.Sc., Biochemistry, 2009)

Supervised by

Prof. Dr. Magdy Mahmoud Mohamed
Professor of Biochemistry
Faculty of Science - Ain Shams University

Prof. Dr. Nabila Anwar El-Sheikh
Professor of Microbiology
Faculty of Medicine for Girls - Al-Azhar University

Dr. Nour Mohamed Abd El-Kader
Lecturer of Biochemistry
Faculty of Science - Ain shams University

Faculty of Science
Ain Shams University
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Mohamed Hasan Mohamed Roshdy

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Has been approved by: **Signature**

1- Prof. Dr. Khalifa El-Sayed Khalifa

Professor of Parasitology,

Faculty of Medicine,

Ain Shams University

2- Prof. Dr. Mahmoud Abdel- Magid Yassin

Professor of Microbiology,

Faculty of Pharmacy,

Ain Shams University

3- Prof. Dr. Magdy Mahmoud Mohamed

Professor of Biochemistry,

Faculty of Science,

Ain Shams University

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ABSTRACT

Abstract

E. histolytica is considered one of the causes that result in mortality and morbidity worldwide through diarrheal diseases and abscess establishment in some tissues such as liver, lung, and brain. The prevalence of *E. histolytica* is different worldwide; several studies have revealed the percentage of that parasite in different geographical areas.

Up till now microscopic examination is still the main way for the diagnosis of amoebiasis. However, light microscopy cannot distinguish between *E. histolytica* and non-pathogenic species as *E. moshkovskii* and *E. dispar* neither in the cyst nor trophozoite stages.

Differential detection of these morphologically indistinguishable protozoan parasites has a great clinical as well as epidemiological importance.

The aim of the present study is to identify and differentiate between *E. histolytica* and *E. dispar* in dysenteric samples from Cairo, Egypt based on molecular techniques.

One hundred and ninety four microscopically diagnosed positive stool samples for *E. histolytica* infection were included in the study from different hospitals and laboratories in Cairo governorate. Genotype analysis of

those samples was performed using a nested multiplex PCR targeting 16S-like rRNA gene to identify *E. histolytica* and *E. dispar* and confirmed by q-PCR targeting SSU-rRNA.

A specific amplified product for *E. histolytica* targeting the 16S-like was detected in (20/194, 10.3%), while *E. dispar* was detected in (17/194, 8.7%). According to the method used in the present study the other negative 157 samples were falsely diagnosed as *E. histolytica*, where the microscopically detected cysts could be for other indistinguishable species such as *E. hartmani*, *E. coli* or any other *Entamoeba sp* that may exist in the human stool.

The results of q-PCR targeting SSU-rRNA gene confirmed the negative results for all 157 samples. In conclusion, there could be an over estimation of the exact prevalence of *E. histolytica* in Egypt. The routine microscopic examination in different laboratories cannot be considered a gold standard technique and should be confirmed by performing molecular or immunological diagnosis.

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List of Abbreviations

ALA.....Amoebic Liver Abscess

CP.....Cysteine Protease

C_T .....Cycle threshold

DNA .....Deoxyribonucleic Acid

dNTPs.....Deoxynucleotide triphosphate

EDTA.....Ethylene Diamine Tetracetic Acid

EhCP5 .....Entamoeba Histolytica Cysteine Protease 5

ELISA .....Enzyme-Linked Immunosorbent Assay

Et Br.....Ethidium Bromide

FAM.....6-Carboxy-Fluorescine

GalNAC.....N-acetyl-d-galactosamine

IFA.....Immunofluorescence Assay

IgM .....Immunoglobulin M

IHA .....Indirect Hemagglutination

MGB.....Minor Groove Binding

NFQ .....Non Fluorescent Quencher

PCR .....Polymenase Chain Reaction

RBCs.....Red Blood Corpuscles

RNA .....Ribonucleic Acid

SAF.....Sodium Acetate Formalin

SREPH.....Serine-Rich E-histolytica Protein

SSU-rRNA .....Small Subunit Ribosomal RNA

TAE .....Tris-Acetate EDTA

TBE .....Tris-Borate EDTA

TPP.....Triage Parasite Panel

UDG.....Uracil-DNA Glycosylase