DETECTION OF G1 GENOTYPE OF HUMAN CYSTIC ECHINOCOCCOSIS IN EGYPT

Thesis Submitted To Faculty Of Medicine Ain Shams University For Partial Fulfillment Of M.D. Degree In Medical Science (Parasitology)

Presented By

Heba Abd-El Kader Ramadan Aminou

M.B., B.Ch., M.Sc., Assistant Lecturer of Medical Parasitology, Faculty of Medicine, Ain Shams University

Supervised By

Professor Doctor/Mohammad Hassan Abd Elbaki

Professor of Medical Parasitology, Faculty of Medicine, Ain Shams University

Professor Doctor/Adel Mohamed Gamal El Missiry

Professor of Medical Parasitology and Executive Director of Medical Research Centre, Faculty of Medicine, Ain Shams University

Professor Doctor/Heba El Sayed Mohamed Abd El Aaty

Professor of Medical Parasitology, Faculty of Medicine, Ain Shams University

Doctor/Anhar Abd El Aziz Mohamad

Assistant Professor of Medical Parasitology, Faculty of Medicine, Ain Shams University

Faculty of Medicine Ain Shams University 2007

الكشف عن المورثة G1 لحالات الأكياس المائية في الانسان في مصر

رسالة مقدمة لكلية الطب جامعة عين شمس توطئة للحصول على درجة الدكتوراه في العلوم الطبية الأساسية (علم الطفيليات)

مقدمة من

الطبيبة/ مبة عبد القادر رمضان أمينو

مدرس مساعد بقسم علم الطفيليات كلية الطب جامعة عين شمس

تحت اشر اف

الأستاذ الدكتور/ محمد حسن على عبد الباقي

استاذ بقسم علم الطفيليات كلية الطب جامعة عين شمس

الأستاذ الدكتور/ عادل محمد جمال المسيري

استاذ بقسم علم الطفيليات المدير التنفيذي لمركز البحوث الطبية كلية الطب جامعة عين شمس

الأستاذ الدكتور/ مرة السيد محمد عبد العاطي

استاذ بقسم علم الطفيليات كلية الطب جامعة عين شمس

الدكتور / انمار عبد العزيز محمد

استاذ مساعد بقسم علم الطفيليات كلية الطب جامعة عين شمس

> كلية الطب جامعة عين شمس 2007

Acknowledgement

I wish to express my deepest gratitude to Prof. Dr. Mohammad H. Abd Elbaki, Professor of Medical Parasitology, Faculty of Medicine, Ain Shams University, for his sincere guidance, valuable advices and support.

Also, I am deeply indebted to Prof. Dr. Adel M. Gamal El Missiry, Professor of Medical Parasitology and Executive Director of Medical Research Centre (MRC), Faculty of Medicine, Ain Shams University, for his precious advices, continuous encouragement and for providing me with all the facilities needed to perform the practical part of this work in the MRC.

I owe a particular gratitude to Prof. Dr. Heba El Sayed M. Abd El Aaty, Professor of Medical Parasitology, Faculty of Medicine, Ain Shams University, for her indispensable advices, valuable suggestions, continuous help and guidance throughout this work.

My special thanks to Dr. Anhar Abd El Aziz Mohamad, Assistant Professor of Medical Parasitology, Faculty of Medicine, Ain Shams University, for her sincere and continuous direction and help.

I owe much appreciation to Dr. Manal Nour in Medical Research Centre (MRC), Faculty of Medicine, Ain Shams University for her great help in conducting the molecular practical part of this work.

Many thanks to all staff members of Medical Research Centre (MRC), Faculty of Medicine, Ain Shams University for their kind help throughout the practical part of this work.

Many thanks to all staff members of Radiodiagnosis Department, Faculty of Medicine, Ain Shams University for their kind help.

Great thanks and appreciation to Prof. Dr. Laila Abd El Halim Al Okby, Professor and Head of Medical Parasitology Department, Faculty of Medicine, Ain Shams University, and all staff members of the Medical Parasitology Department, for their sincere help.

Last but not least, I wish to express my gratitude and appreciation to my dear parents and husband who always encourage and support me throughout my life.

Contents

	Page No.
Introduction	1
Review of literature	5
* Classification of Echinococcus granulosus	5
* Geographical distribution, prevalence and epidemiology	6
* Morphology and life cycle	12
* Immunology and immunopathogenesis of cystic echinococcosis	22
A. Immune mechanisms	22
 Innate resistance and early immunity Immunity against established cysts 	22 24
B. E.granulosus antigens	31
* Pathology, clinical picture and complications	36
*Diagnosis of cystic echinococcosis	43
[1] Clinical picture	43
[2] Immunological techniques	43
A. Tests for antibody detection	45
B. Tests for free and/or immune complexed hydatid	62
specific antigens	02
C. Cell mediated immunological tests	65
[3] Non immunologic methods	67
A. Imaging techniques	68 71
B. Closed aspiration of the cyst C. Riochemical and hematological tests	71 73

* Molecular biology in the diagnosis of cystic echinococcosis	74
(1) DNA isolation	76
(2) Restriction endonucleases	77
(3) Nucleic acid hybridization and probes	78
(4) Detection of genes	80
a. In-situ hybridization	80
b. Fluorescence in-situ hybridization	80
c. Blotting techniques	81
(5) DNA sequencing	82
(6) DNA amplification	87
* Polymerase Chain Reaction (PCR)	89
(1) Qualitative PCR	93
a. Nested PCR	93
b. Reverse transcriptase PCR	94
c. Multiplex PCR	94
d. Ligase chain reaction	95
e. In situ PCR	95
(2) Quantitative PCR	96
a. Quantitative competitive reverse transcriptase	96
PCR	
b. Real time PCR	96
PCR application in hydatid disease	98
*Echinococcus granulosus strains	102
-Strains	103
- Approaches for <i>E.granulosus</i> strain characterization	115
Aim and plan of the work	121
Subjects and methods	124
Results	170

Discussion	215
Summary and conclusions	237
Recommendations	245
References	246
Arabic summary	

List of Figures

		Page No.
Figure (1)	Geographical distribution of CHD	11
Figure (2)	Life cycle of Echinococcus granulosus	12
Figure (3)	The whole worm	13
Figure (4)	The scolex	13
Figure (5)	Hydatid sand	20
Figure (6)	DNA structure	76
Figure (7)	Southern blotting	81
Figure (8)	Steps of polymerase chain reaction	91
Figure (9)	Real time PCR	98
Figure (10)	Echinococcus granulosus protoscoleces found in human hydatid cyst fluid	131
Figure (11)	US scan showing simple, rounded, well circumscribed hydatid cyst in the liver	136
Figure (12)	US scan showing signs of degeneration of the laminated membrane of a hydatid cyst found in the liver	137
Figure (13)	DNA extraction kit	148
Figure (14)	The pouring of the horizontal agarose gel	165
Figure (15)	Mean age in the six studied groups	175
Figure (16)	Percentage of sex distribution in	175

	the six studied groups	
Figure (17)	Distribution of CE patients according to	178
	the affected organ	1/0
Figure (18)	Distribution of CE patients according to	179
	the size of the cyst	1/9
Figure (19)	Frequency of symptoms in patients with	181
	cystic echinococcosis (CE)	
Figure (20)	Results of IHAT in the six studied groups	184
Figure (21)	Plate showing the positive and negative reactions of IHAT (indirect haemagglut-ination test)	185
Figure (22)	Relation between result of IHAT and sex	187
Figure (23)	IHAT titre in the six studied groups	190
Figure (24)	Sensitivity and specificity of IHAT in relation to hydatid cyst fluid examination in the diagnosis of cystic echinococcosis	192
Figure (25)	Results of PCR in hydatid fluid from different animal isolates	194
Figure (26)	Results of PCR in hydatid fluid from patients (human isolates) with CE	196
Figure (27)	Results of PCR in hydatid fluid on 1% agarose gel electrophoresis stained with ethidium bromide	197
Figure (28)	Results of PCR in sera of individuals in the six studied groups	200
Figure (29)	Results of PCR in sera of individuals in the different groups on 1% agarose gel electrophoresis stained with ethidium bromide	201
Figure (30)	Relation between results of PCR serum	203

and sex

Figure (31)	Sensitivity and specificity of PCR serum in relation to hydatid cyst fluid examination in the diagnosis of cystic echinococcosis	205
Figure (32)	Results of IHAT for hydatid disease and PCR in sera of patients in group IV (parasitic control group)	211
Figure (33)	Results of IHAT for hydatid disease and PCR in sera of patients in group V (other mass occupying lesions)	213

List of Tables

		Page No.
Table (1)	Echinococcus granulosus species, strains and genotypes	104
Table (2)	Descriptive data for the selected cases in the study regarding age and sex	174
Table (3)	Frequency and percentage of the different	177

	sites (organs) involved in the 36 CE	
	patients	
Table (4)	Frequency and percentage of the size of cysts identified in the 36 CE patients	179
Table (5)	Frequency of symptoms in patients with	180
	cystic echinococcosis (CE)	
Table (6)	Results of IHAT in the six studied groups (using Pearson Chi-Square)	183
Table (7)	Relation between results of IHAT and sex	186
Table (8)	IHAT titre in the six studied groups	189
Table (9)	The validity of IHAT in relation to hydatid cyst fluid examination in the diagnosis of cystic echinococcosis	191
Table (10)	Results of PCR in hydatid fluid from different animal isolates	193
Table (11)	Results of PCR for detection of G1 genotype in hydatid fluid from patients (human isolates) with CE	195
Table (12)	Results of PCR in sera of individuals in the six studied groups (using Pearson Chi- Square)	199
Table (13)	Relation between results of PCR serum and sex	202
Table (14)	The validity of PCR serum in relation to hydatid cyst fluid examination in the diagnosis of cystic echinococcosis	204
Table (15)	Comparison between results of PCR for detection of G1 genotype of <i>E. granulosus</i> metacestodes in both human hydatid fluid (human isolates) and sera of patients with CE	207
Table (16)	Comparison between results of PCR (in	208

	both human hydatid fluid and sera) and frequency of symptoms concerning patients with CE	
Table (17)	Results of IHAT for hydatid disease and PCR in sera of patients in group IV (parasitic control group)	210
Table (18)	Results of IHAT for hydatid disease and PCR in sera of patients in group V (other mass occupying lesions)	212
Table (19)	Comparison between results of IHAT and PCR in sera of patients in the six studied groups	214

Abbreviations

A Adenine
Ab Antibody

AE Alveolar echinococcosis

Ag Antigen

AHD Alveolar hydatid disease

bp Base pair

Buffer AE Elusion buffer
Buffer AL Lysis buffer

Buffer Aw1 Washing buffer 1

Buffer Aw2 Washing buffer 2

C Cytosine

CAg Circulating antigen

CE Cystic echinococcosis

CFT Complement fixation test

CHD Cystic hydatid disease

CICs Circulating immune complexes

CIEP Counter-immunoelectrophoresis

CT Computerized tomography

DD5 Arc-5 Double Diffusion

ddH2O Doubled distilled water

DH Definitive host

DNA Deoxyribonucleic acid

DNase Deoxyribonuclease

E.granulosus Echinococcus granulosus

E.multilocularis Echinococcus multilocularis

ELISA Enzyme linked immunosorbent assay

FISH Fluorescence in-situ hybridization

FNAB Fine needle aspiration biopsy

G Guanine

HCF Hydatid cyst fluid

IDT Intradermal test

IFAT Indirect immunofluorescent antibody test

Ig Immunoglobulin

IH Intermediate host

IHAT Indirect haemagglutination test

ISH In-situ hybridization

KDa Kilo Dalton

LAT Latex agglutination test

LCR Ligase chain reaction

MRI Magnetic resonance imaging

PAIR Percutaneous aspiration injection

reaspiration

PCR Polymerase chain reaction

QCRT-PCR Quantitative competitive reverse

transcriptase PCR

RAPD-PCR Random amplified polymorphic DNA-PCR

RAST Radioallergosorbent test

REs Restriction endonucleases

RFLP Restriction fragment length polymorphism

RNA Ribonucleic acid

RT-PCR Reverse transcriptase PCR

SDS-PAGE Sodium dodecyl sulfate polyacrylamide gel

electrophoresis

T Thymine

U/S Ultrasound

WB Western blotting

Introduction

Human echinococcosis is a disease that results from parasitism by the larval stage of four *Echinococcus* species of which *Echinococcus granulosus* (*E.granulosus*) causing cystic hydatid disease and *E.multilocularis* causing alveolar hydatid disease are the most important. Minor species are *E.vogeli* causing polycystic hydatid disease and *E.oligarthrus* (Goldsmith et al., 2005).

Cystic echnicoccosis (CE), termed "hydatid disease" or "hydatidosis" is caused by infection with the larval stage (metacestode) of the dog tapeworm *E.granulosus*. It is a major zoonosis of worldwide distribution and is especially prevalent in sheep raising countries (McManus et al., 2003). The disease is characterized by long – term growth of metacestode cysts in humans and domestic animals. It is not only important as a public health problem in areas where the disease is endemic, but also responsible for significant economic loss in livestock (Li et al., 2003).

CE is considered as an emerging disease in various regions, e.g. the Middle East, Central Asia, and Northern and Eastern Africa (Eckert et al., 2001).

Now, it is well recognized that *E.granulosus* exhibits extensive intraspecific (strain) variation that may impact the diagnosis, epidemiology, pathology and control of hydatid disease. It has also important implications for the design and