Evaluation of the Diagnostic Efficacy of a Panel of Monoclonal Antibodies Developed against *Fasciola gigantica* Antigens

THESIS

Submitted for Fulfilment of Master Degree (M. Sc.)
in
Clinical Pathology

By

Engy Mohsen AbdEl-Moneem
M.B.B.CH
Theodor Bilharz Research Institute

SUPERVISORS

Prof. Dr. Taghred Mohamed Gaafr
Professor of Clinical Pathology & Immunology
Cairo University

Prof. Dr. Zeinab Abo-Bakr Demerdash
Professor of Clinical Pathology & Immunology
Theodor Bilharz Research Institute

Faculty of Medicine
Cairo University
(2009)

Acknowledgments

''First and foremost thanks are due to God
The Beneficent and the Merciful''

It is great to feel success and have the pride of achieving all what is always aspired. Nevertheless, one must not forget all those who usually help and push him onto the most righteous way that inevitably ends with fulfillment and perfection.

I wish to express my deep appreciation and profound gratitude to **Prof. Dr. Taghrid Mohamed Gaafar**, Professor of Immunology, Faculty of Medicine, Cairo University, for her excellent guidance, valuable suggestions and unfailing support.

When the instant comes to appreciate all those kind-hearted people, I soon mention **Prof. Dr. Zeinab Abo Bakr Demerdash**, Professor of Immunology, Theodor Bilharz Research Institute, the person who gave me the honor to be her student. She really helped me by her precious opinions, extremely valuable scientific suggestions and contributive comments that served much in the construction of this work.

Great thanks are due to **Prof. Dr. Faten Salah El Din Mahmoud,** Professor of Immunology, Theodor Bilharz Research Institute. She was always there to care, support, encourage and provide constructive pieces of advice in every possible way. She really inspires me.

Thanks also to **Prof. Dr. Salwa Hassan Mohamed**, Professor of Immunology, Theodor Bilharz Research Institute, who has been the real sister, thanks for her great concern and close supervision in the practical part of the work.

A special tribute is paid to **Mrs. Houda Abou-Taleb,** assistant lecturer in Environment Research Department, Theodor Bilharz Research Institute, for her generous effort and her creative thinking during the statistical analysis of the study.

I would also like to record endless and forever love to my **family and husband to** whom i`m honored to belong.

I will not forget my collueges at Immunology Laboratory, Theodor Bilharz Research Institute as their help was beyond my imagination.

Engy Mohsen

2009

Abstract

The development of a MAb-based sandwich ELISA for detection of Fasciola antigen in sera and stool samples of fascioliasis patients to provide a sensitive and reliable method for immunodiagnosis of active fascioliasis.

Key Words : Monoclonal Antibodies Developed against *Fasciola*

Table of Contents

List of Abbreviations

Ab	Antibody.
Ag	Antigen.
BSA	Bovine serum albumin.
cDNA	Comlementary deoxyribonucleic acid.
CFT	Complement fixation test.
CIE	Counter immunoelecterophoresis.
CL1	Cathepsin L like 1.
CL2	Cathepsin L like 2.
DEAE	Diethyl aminoethanol.
DMSO	Dimethyl sulfoxide.
DW	Distillled water.
ELISA	Enzyme linked immunosorbent assay.
EITB	Enzyme linked immunoelectrotransfer blot.
EPD	Electronic pulse delivery.
EPs	Excretory products.
E/S	Excretory/secretory.
FAST	Falcon assay screening test.
FCS	Fetal calf serum.
F. gigantica	*Faciola gigantica.*
F. hepatica	*Fasciola hepatica.*
GLDH	Glutamate dehydrogenase.
HAT	Hypoxanthene aminopterine and thymidine.
HRP	Horse-radish peroxidase.
ICC	Immunocytochemistry.
ID	Immunodiffusion.
IEM	Immunoelectron microscopy.
IgA	Immunoglobulin A.
IgE	Immunoglobulin E.
IgG	Immunoglobulin G.
IgM	Immunoglobulin M.
IHA	Indirect heamagglutination test.
IHC	Immunohistochemistry.
IIF	Indirect immunofluorescence.
IL1	Interleukin-1.
IL4	Interleukin-4.
kDa	kilo Dalton.
LAT	Latex agglutination test.
LSD	Least significant difference.
mA	Milliampere.
MAb	Monoclonal antibody.
MIFC	Merthiolate iodine formaldehyde concenteration.

mM	Millimole.
mRNA	Messenger ribonucleic acid.
OD	Optical density.
OPD	Ortho-phenylenediamine.
PBS	Phosphate buffer saline.
PCR	Polymerase chain reaction.
PEGs	Polyethylene glycols.
PGH	Porcine growth hormone.
RAPD	Random amplified polymorphic cDNA.
RNA	Ribonucleic acid.
ROS	Reactive oxygen hormone.
RPMI	Rosewell Park Memorial Institute.
R.p.m	Round per minute.
scFv	Single chain variable fragment.
SD	Standard deviation.
SEM	Standard error of the mean.
SBSC-TBRI	*Schistosoma* Biological Supply Center, Theodor Bilharz Research Institute.
SDS-PAGE	Sodium dodecyl sulphate- polyacrylamide gel electrophoresis.
SFM	Serum-free media.
SGPT	Serum glutamic pyruvic transaminase.
SGOT	Serum glutamic oxaloacetic transaminase.
S.haematobium	*Schistosoma hematobuim.*
S. mansoni	*Schistosoma mansoni.*
T	Tween.
T1	Tegument 1.
T2	Tegument 2.
TCBZ	Triclabendazole.
Th	T-helper cell.
Th2	T-helper 2 cell.
WHO	World Health Organization.
X	Mean.

List of Tables

List of Figures

Introduction

Introduction

Liver fluke disease “fascioliasis” is an important parasitic disease found worldwide affecting sheep, goats, cattle and buffalos, as well as other domestic ruminants. In cattle, sheep and goats, fascioliasis disease is responsible for serious economic loss (Sexton *et al*., 1991). Between 1970 and 1990, human fascioliasis was considered a secondary disease by public health officials, with only approximately 2,000 cases reported. In recent years; fascioliasis can no longer be considered merely a secondary zoonotic disease, but an important human parasitic disease, with approximately 2.4 to 17 million infected people (Ashrafi *et al*., 2004).

In Africa, most human cases were reported in Egypt, especially in Nile Delta (Mas-Coma *et al*., 2005). The pathogenicity of fascioliasis in humans has been recognized, the number of cases reported has increased in 51 countries in five continents (Mas-Coma *et al*., 1999; Esteban *et al*.,1998). Areas endemic for human disease that are not necessarily related to those endemic for animal disease have been described, in which fascioliasis chronicity and superimposed repetitive liver fluke infections posed additional pathologic complications (Mas-Coma *et al*., 1999). Juvenile flukes penetrate the intestinal wall, then the flukes migrate within the abdominal cavity and penetrate the liver or other organs and ectopic location of flukes can occur (Chen and mott, 1990; Behm and Sangster, 1999). Fibrosis of the liver is the main cause of pathogenesis in fasioliasis patients. The major pathological changes are seen during migration of the immature flukes through the liver parenchyma before they enter the biliary tree (Adel, 2000), then pass into the biliary tract where they reach maturity leading to

hypertrophy of biliary ducts associated with obstruction of the lumen.

Diagnosis of fascioiasis, schistosomiasis and other parasitic disease in endemic areas depends mainly on microscopic detection of eggs in the stool or urine (Barreto *et al*., 1990).

Patients infected with *Fasciola* may escape diagnosis because of the difficulties in detecting eggs in stools (Chen and Mott, 1990; Hillyer 1988). Moreover, *Fasciola* eggs may be found in the stool of uninfected persons who have eaten raw infected liver, leading to false positive diagnosis (Bhamarapravati *et al*., 1983; Hillyer, 1999).

Humans and experimental animals develop a complex array of humoral and cellular immune responses during the course of infection. During clinical and parasitological investigations, false negative results are common especially in light infections, leading to failure of diagnosis of many infected cases and to a strong underestimation of the prevalence of the disease (De -Vias *et al*., 1992).

Immunodiagnostic assays for detecting anti-*Fasciola* antibodies, although considered useful for diagnosing fascioliasis, did not discriminate between previous exposure and current infection (Simpson and Smithers, 1985; Espino and Finlay, 1994; Mezo *et al.*, 2004).

Antigen detection assays seem to be more effective in determining active infection and hence the efficacy of chemotherapy (Maleewong *et al*.,

1997b). Furthermore, circulating antigen detection could be a good indicator of intensity of infection (Barsoum *et al*., 1991; Van Lieshout *et al*., 1995).

Increased interest has been shown in the use of MAbs in antigen detection assays because of their ability to recognize specific antigens with precise determinations and their homogeneity (Zodda *et al*., 1983). The use of MAbs has greatly improved the sensitivity of assays used in the detection of circulating *Fasciola* antigens (Rudbach *et al*., 1995). The antigen detection assays have been greatly improved to overcome the difficulties in diagnosing light and recent infections as well as successful monitoring of cure of fascioliasis disease (Maleewong *et al*., 1997b).

The use of sandwich enzyme-linked immunosorbent assay using monoclonal antibodies (MAb) to detect *Fasciola* specific execratory/ secretory (ES) antigens in stool specimens of patients infected with fascioliasis was considered an accurate, effective method for diagnosis of active infection (Shi *et al*., 1991; Espino *et al*., 1990 and 1998).

Aim of the work

Aim of the work

1- Screening and evaluation of MAbs, produced by culture of already available hybridoma cell lines against crude and execretory/ secretory *Fasciola* antigen (ESA) produced at Immunology Dept, TBRI; and preserved in liquid nitrogen.

2- The development of a MAb-based sandwich ELISA for detection of E/S *Fasciola* antigen in sera and stool samples of fascioliasis patients to provide a sensitive and reliable method for immunodiagnosis of active fascioliasis.

Review of literature