Ain-Shams University Faculty of Science Zoology Department



## COMPARATIVE STUDY OF SENSITIVITY OF SOME DIAGNOSTIC METHODS FOR DETECTING EARLY SCHISTOSOMA MANSONI INFECTION IN MICE

A Thesis
Submitted for the Award of Ph.D. in Zoology
(Molecular Biology)

By

#### REHAB MAMDOUH ABDEL-MEGEED MOSTAFA

Assistant researcher at National Research Center B.Sc., Zoology, (2001) M.Sc., Molecular biology(2005) TO

Zoology Department - Faculty of Science,

Ain-Shams University

### **Supervision committee:**

#### Prof. Dr. Abdalla Mohamed Ibrahim

Professor of Malacology, Faculty of Science, Ain - Shams University, Cairo, Egypt

#### Prof. Dr. Maha Zaki Rizk

Professor of Biochemistry, Theraputical Chemistry

Department, National Research Center, Cairo, Egypt.

#### **Prof. Dr. Amany Sayed Maghraby**

Professor of Immunology and parasitic disease, Therapeutical Chemistry Department, Immunology and Infectious Diseases group, Center of Excellence for Advanced Sciences, National Research Center, Cairo, Egypt

#### Dr. Mahmouh Mohamed Bahgat

Assistant Professor of molecular Immunology, Therapeutical Chemistry Department, Immunology and Infectious Diseases group, Center of Excellence for Advanced Sciences, National Research Center, Cairo, Egypt

## APPROVAL SHEET

#### **Prof. Dr. Sanaa Thabet Botros**

Professor of Immunology Pharmacology Department, Theodor Bilharz Research Institute, Cairo, Egypt

#### Prof. Dr. Al Sayed Mohamed Mahdy

Professor and head of biochemistery Department, Faculty of Science - Hillwan University - Cairo, Egypt.

#### Prof. Dr. Abdalla Mohamed Ibrahim

Professor of Malacology, Faculty of Science, Ain - Shams University, Cairo, Egypt

#### Prof. Dr. Maha Zaki Rizk

Professor of Biochemistry, Theraputical Chemistry

Department, National Research Center, Cairo, Egypt

#### **ABSTRACT**

Schstosomiasis is considered as the second most important human parasitic disease after malaria causing morbidity and mortality. This morbidity and mortality is associated with the chronic stage of infection. Thus a diagnostic tool capable of detecting Schistosoma infection in the acute phase would be of great value. In the present work two Cercarial and two worm antigen preparations were used to detect IgG in plasma of S. mansoni infected mice by Enzyme linked immunosorbent assay (ELISA). In parallel, specific primers for the parasite genome were used to detect infection in plasma & urine from infected mice. Results showed that all the above mentioned diagnostic approaches enabled detecting infection as early as 3 days post mice exposure to parasite. Detection of parasitic DNA in urine samples was the most sensitive and specific test for detecting infection than all the above mentioned tests. Furthermore the study extended to detect the infection in the snail's tissue at prepatancy and patancy stages using polymerase chain reaction (PCR). Results showed 100% sensitivity and specificity of such technique.

The present work was conducted to detect infection in the main host using antigens extracted from an intermediate snail host by ELISA. The result illustrated higher IgG reactivity with both snails' tissue homogenate and hemolymph of infected snails reflecting presence of common antigens between the parasite and its snail intermediate host.

١



# **ACKNOWLEDGMENT**

First and above all, I would like to express my great thanks to Allah, for helping me to overcome all problems, which faced me throughout the work.

My sincerest appreciation and deep gratitude to **Prof. Dr.**Abdalla Mohamed Ibrahim, Professor of Malacology, Zoology
Department, Faculty of Science, Ain Shams University, for his
continuous guidance, encouragement and his generous help to
complete this work as well as valuable efforts in reviewing the
text.

Words cannot express my sincere thanks and appreciation to **Prof. Dr. Maha Zaki Rizk,** Professor of Biochemistry, Theraputical Chemistry Department, National Research Center, for her beneficial supervision and guidance throughout the different phases of the work and for her valuable efforts in reviewing the text.

I am also deeply grateful to **Prof. Dr. Amany Saiyd Maghraby,** Professor of immunology and parasitic disease,
Therapeutical Chemistry Department, Immunology and Infectious
Diseases group, Center of Excellence for Advanced Sciences,
National Research Center for her help in experimental part and for
her valuable efforts in reviewing the text in addition to her kind
help and continuous interest.

Words cannot express my sincerest appreciation and deep gratitude to **Dr. Mahmoud Mohamed Bahgat** Assistant professor of Molecular immunology and parasitic diseases, Therapeutical Chemistry Department, Immunology and Infectious Diseases group, Center of Excellence for Advanced Sciences, National Research Center for his support throughout different phases of the wok. All of thanks for his help in experimental part, his valuable efforts in reviewing the text, providing all facilities to complete this work and for his guidance that helped me to overcome all the problems throughout the work.

Also I wish to express my deep thanks to my supervisor in master thesis **Prof. Dr. Abdel-Hamid Zaki Abdel-Hamid,** Professor of Biochemistry and Molecular Biology, theraputical Chemistry Department, National Research Center for his support, kind help and guidance

All of thanks for my colleges **Hanaa Gaber**, **Rabeh Al Shesheny** and **Karim Awad** for their help in an experimental part.

I would like to thank all members of theraputical Chemistry Department and Infectious Diseases group, Center of Excellence for Advanced Sciences, National Research Center, for their encouragement, help, and for the facilities provided.

> The author Rehab Mamdouh

# List of Contents

Item	Page
Acknowledgment	
Abstract	
List of Tables	
List of Figures	
List of Abbreviations	_
ntroduction	1
Aim of the work	4
Review of Literature	5
- Global and national situations of human schistosomiasis	5
- Parasite life cycle	6
Diagnosis of infection	10
1- Parasitological	10
2- Immunodiagnosis	11
A Detection of parasite circulating antigens	11
B Detection of anti-parasite antibodies	14
· Comparing the diagnostic values of antigen and antibody detections.	18
3- Molecular diagnosis	20
1. Diagnosis through main host	20
2. Diagnosis through an intermediate host	22
· Comparing sensitivity and specificity between PCR and the	
previously mentioned diagnostic tools	24
	4
· Homology between antigenic titer excreted from <i>S. mansoni</i> worms	
and intermediate snail host	27
Material and Methods	31
- Materials	31
Animals and parasites	31
Maintenance of mice	31
Maintenance of snails	31
· S. mansoni life cycle	31
· Infected mice groups and samples collection	33
Collected samples from infected snails	34
• Reagents and solutions	34
1. Anti-coagulant (1.5% w/v)	34
2. Reagents used in ELISA	34

<ul><li>3. Buffers and reagents for gDNA extraction</li><li>4. Reagents and stock solutions used for agaro</li></ul>	
gel electrophoresis	
PCR reaction mixture	
Methods	
1. Preparation of antigens	
<ol> <li>Detection of IgG levels by ELISA in plasm mice infected with <i>S. mansoni</i> cercariaea against SWAP, CS and WoV</li></ol>	st CAP, from S.
tissue homogenates	
4. gDNA extraction	
6. Bioinformatics analysis of the used primers	
7. Amplification of genomic DNA	
8. Statistical analysis	
1. Detection of IgG levels by ELISA in plasma from S. mansoni infected mice	AP, CS,
SWAP and WoV at early versus late time points post S. I	
infection	
<ul><li>4. Percentages IgG cross reactivity in individual IMP amor CS, SWAP and WoV</li><li>5. Cross reactivity of antigens prepared from <i>B. alexandri</i></li></ul>	
with plasma from <i>S. mansoni</i> infected mice	
<ul><li>6.1. Purity and integrity of extracted genomic DN different parasite stages.</li><li>6.2. Prediction of the annealing position and PCR pro</li></ul>	
by the online available bioinformatics tools	ne gDNA
primers	-

### LIST OF CONTENTS

	6.4. PCR amplification of the target S. mansoni sequence on	
	genomic DNA extracted from plasma of mass infected	
	hamster	<b>67</b>
	6.5. PCR amplification of the target S. mansoni sequence	
	from genomic DNA extracted from IMP	68
	6.6. PCR amplification of the S. mansoni sequence from	
	gDNA extracted from infected mice urine samples	<b>70</b>
	6.7. PCR amplification of the S. mansoni sequence from	
	gDNA extracted from infected <i>B. alexandrina</i> homogenate	71
Discussion		72
Summary	•••••	88
•	••••••	92
	mary	I i

## List of Tables

<b>Table</b>		Page
No.		C
1	Detection of IgG reactivity at different time intervals post <i>S.mansoni</i> infection against different antigens	55
2	Comparing IgG reactivity in individual IMP against CAP, CS, SWAP and WoV at early versus late time points post infection	56
3	Cross reaction of CAP IgG positive individual samples against CS, SWAP and WoV	58
4	Cross reaction of CS IgG positive individual samples against CAP, SWAP and WoV	59
5	Cross reaction of SWAP IgG positive individual samples against CAP, CS and WoV	60
6	Cross reaction of WoV IgG positive individual samples against CAP, CS and SWAP	61

## List of Figures

## Figure No.

		Pa
1	Schistosomiasis life cycle with morphologically distinct stages occupying several ecological niches	ge 9
2	Detection of IgG levels by ELISA in <i>S. mansoni</i> infected mice plasma (IMP) Using CAP coated plates	49
3	Detection of IgG levels by ELISA in <i>S. mansoni</i> infected mice plasma (IMP) Using CS coated plates	
4	Detection of IgG levels by ELISA in <i>S. mansoni</i> infected mice plasma (IMP) Using SWAP coated plates	<ul><li>50</li><li>51</li></ul>
5	Detection of IgG levels by ELISA in <i>S. mansoni</i> infected mice plasma (IMP) Using WoV coated plates	52
6	Serial dilutions of IMP (1/250, 1/500 and 1/1000) showed higher IgG reactivities with both snails' tissue homogenate and hemolymph of infected <i>B. alexandrina</i> snails	62
7	Representative figure used to illustrate higher IgG reactivities with both snails' tissue homogenate and hemolymph of infected snails (1/250)	63
8	Electrophoresis film of extracted genomic DNA from <i>S. mansoni</i> adult worms, schistosomula, cercariae, infected <i>B. alexandrina</i> snail tissues (source for parasite sporocysts) as well as hemolymph.	64

### LIST OF FIGURES

9	Deduced target <i>S. mansoni</i> sequence for the PCR amplification using the previously published primer	65
10	Electrophoresis film of amplification products from genomic DNA of adult <i>S. mansoni</i> worms, schistosomula, cercariae, infected <i>B. alexandrina</i> snail tissues and hemolymph	66
11	Electrophoresis film of amplification products from genomic DNA of chronic infected hamster plasma	67
12	Electrophoresis film of amplification products from genomic DNA of early IMP at different time points post infection.	69
13	Electrophoresis film of amplification products from genomic DNA of early infected mice urine at different time points post infection	70
14	Electrophoresis film of amplification products from genomic DNA of exposed and shedding infected <i>B. alexandrina</i> homogenate	71

# List of Abbreviations

AWA	Adult worm antigen
B. aexandrina	Biomphalaria aexandrina
B. glabrata	Biomphalaria glabrata
B. truncatus	Bulinus truncates
CAA	Circulating anodic antigens
CAP	Cercarial antigen preparation
CCA	Circulating cathodic antigens
CE	Cercarial elastase
CMS	Control mice sera
CS	Cercarial secretion antigen
СТАВ	Hexadecyltrimethyl-ammonium bromide
ddH <sub>2</sub> O	Double distilled water
EDTA	Ethylene Diamine Tetra Acetic Acid
ELISA	Enzyme linked immunosorbent assay
ES	Excretory-secretory
FCS	Fetal calf serum
gDNA	Genomic DNA
IFN-γ	Interferon –Gamma
IgG	Immunoglobulin type G
IMP	Infected mice plasma
IMS	Infected mice sera
IRC	Irradiated cercariae

### LIST OF ABBREVIATION

MP	Mean percentage
PBMCs	Peripheral blood mononuclear cells
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PZQ	Praziquentel
RA	Radiation Attenuated
S. heamatobium	Schistosoma heamatobium
S. intercalatum	Schistosoma intercalatum
S. japanicum	Schistosoma japoanicum
S. mansoni	Schistosoma mansoni
S. mekonji	Schistosoma mekonji
SBgA	soluble crude Biomphalaria glabrat
	antigen
SDS	Sodium Dodecyle Sulphate
SEA	Soluble egg antigen
SWAP	Soluble worm antigen preparation
TEMED	N, N, N`, N`-tetramethylethylendiamine
UIMP	Un infected mice plasma
UIMS	Un infected mice sera
WoV	Worm vomit