# The pathologic significance of hysteroscopic diagnosis in cases of premenopausal and postmenopausal bleeding

**Thesis**

**Submitted by**


## Nahla Hashem Ali Ahmed

M.B.B.Ch.

(Resident of Obstetrics and Gynecology in Ghamra Military Hospital)


**For fulfillment of M.Sc. degree**

**Supervised by**


## Prof. Ahmed Lotfy Aboul Nasr

Professor of Obstetrics and Gynecology

Faculty of Medicine, Cairo University

## Dr. Ahmed Soliman Nasr

Assistant Professor of Obstetrics and Gynecology

Faculty of Medicine, Cairo University

## Dr. Sherif Sameh Zaki

Lecturer of Obstetrics and Gynecology

Faculty of Medicine, Cairo University



**Faculty of Medicine**

**Cairo University**

**2012**

# Abstract

Schistosomiases, the major public health problem in rural Egypt, with almost six million people were infected so the early diagnosis is essential for adequate treatment of acute infection. In this study, we compare PCR and Western blot techniques in diagnosis of schistosomiasis *mansoni.* Forty Swiss albino mice were used, thirty two mice were infected with cercariae of *S. mansoni* and eight mice were kept non infected which were used as a control. Blood was obtained from four infected mice weekly beginning from the 1$^{st}$ week to the 8$^{th}$ week post infection. PCR and Western blot techniques were performed on this blood. We found that PCR was positive from the first week post infection and the strips appeared at 110 pb, while Western blot technique was positive from the second week post infection and the antibodies reaction against protein bands appeared at 130, 95, 55, 43 and 32KDa. From these results, we found that PCR can diagnose schistosomiasis *mansoni* earlier than Western blot technique.

Key words: *Schistosoma mansoni*- PCR- Western blot technique.

# *Acknowledgement*

*I am thankful to* ***GOD*** *for all his kindness and grace, for having granted me the patience to accomplish this work.*

*I am extremely obliged to* ***Prof. Dr. Azza Ebrahem El-Adawy,*** *Head of Parasitology Department, Faculty of Medicine, Cairo University, for her encouragement, support and advice.*

*I am truly grateful to* ***Prof. Dr. Olfat Mohammed Al-Matarawy,*** *Professor of Parasitology, Cairo University, for her encouragement and kind support.*

*I feel very grateful to* ***Prof. Dr. Maysa Mohammed Kamel,*** *Professor of Parasitology, Cairo University, for teaching me how to do a scientific research. I am proud to have her as my supervisor in M.Sc.*

*Sincere gratitude and deepest appreciation to my supervisor* ***Prof. Dr. Mousa Abdel Gawad Mousa,***

*Professor of Parasitology, Cairo University, for his Keen, co-operation, guidance, continuous encouragement and enlightening vision from the early moment of the study till presentation of this work. His meticulous revision of the work had helped me so much. I am proud to have hem as my supervisor.*

*I am truly grateful to* ***Prof. Dr. Wahed Mohammed Ali Mousa,*** *Professor of Parasitology, Cairo University, not only trained me on the scientific research methods, but also helped and supported me during this study. He offered me unremitting care and guidance from the early moment of the study till presentation of this work. His valuable supervision during the preparation of this thesis will remain a valued memory.*

*Grateful as can be to* ***Dr. Enas Yahia Abu Sarea Mohamed*** *Lecturer of Parasitology, Beni Suief University, Through her patience, insight and vast*

*knowledge who helped in bringing this research into existence. Without her meticulous super vision, the present work could not be accomplished.*

*Words can not express my deepest gratitude for* ***Dr. Maha Mohammed Abou El-Magd*** *lecturer of parasitology- Cairo university who gave me the opportunity to do this work under her sincere guidance, encouragement and eminent super vision.*

*Special thanks for all the staff and doctors of Department of Parasitology, Faculty of Medicine, Cairo University, for their help and support during the present study.*

*I want to thank* ***my mother, my father, my husband, my daughters and my son*** *for their never ending support and ever lasting care and help. Without their support, I would never make any achievement in my life.*

# Contents

# List of tables

# List of figures

# List Of Abbreviation

| | |
|---|---|
| **Bp** | **Base pair.** |
| **CFT** | **Complement fixation test.** |
| **CIEP** | **Counterimmunoelectrophoresis test.** |
| **COI** | **Cytochrome oxidase I.** |
| **COPT** | **The circumoval precipitation test.** |
| **ddNTPs** | **dideoxynucleotide triphosphates.** |
| **EITB** | **Enzyme linked Immunotransfer Blot.** |
| **ELISA** | **Enzyme linked immunosorbant assay.** |
| **FDA** | **Fast dot-enzyme-linked immunosorbent assay.** |
| **GAA** | **Gut Associated Antigen.** |
| **IFAT** | **Indirect immunofluorescent Antibody test.** |
| **IHAT** | **Indirect haemaglutination test.** |
| **ITS** | **Intergenic Spacer Regions.** |
| **KDa** | **Kilo Dalton.** |
| **M** | **Mol.** |
| **MBA** | **Membrane Bound Antigen.** |
| **N.C.** | **Nitrocellulose.** |
| **PBS** | **Phosphate Buffered Saline.** |
| **PCR** | **Polymerase Chain Reaction.** |
| **RFLP** | **Restriction Fragment Length Polymorphism.** |
| **S. mansoni** | **Schistosoma mansoni.** |
| **SDS–PAGE** | **Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis.** |
| **SEA** | **Soluble egg antigen.** |
| **Sm** | **Schistosoma mansoni.** |
| **TCA** | **Trichloroacetic acid.** |
| **UV** | **Ultraviolet.** |
| **V** | **Volt** |
| **WA** | **Worm Antigens.** |
| **WHO** | **World Health Organization.** |

# Introduction

Schistosomiasis is a major parasitic disease that affects more than 200 million people worldwide approximately, 20 million of them have symptoms and 20 million have severe illness, with more than 600 million people in the tropics are at risk for developing Schistosomiasis **(Waine and Mc Manus, 1997).**

Schistosomiasis is one of the major public health problem in rural Egypt, with almost six million people are infected **(El-Khoby *et al*., 1998).** The presence of Aswan Dam has led to the virtual elimination of *S. heamatobium* from the Nile Delta but has brought the establishment of *S. mansoni* in Upper Egypt **(Patz *et al*., 2000).**

Acute Schistosomiasis may commonly develop in the immunologically negative host. Six to eight weeks after the initial contact with water infected by cercaria, clinical manifestation of the disease appear and they vary in intensity from relatively mild to severe **(Rabello, 1992).** Heavy infections contribute to anaemia and can retard children's growth, physical activity and congestive function. If such infections left untreated, they will lead to substantial morbidity, including peripheral hepatic fibrosis **(Richter *et al*., 2003).**

The current method for diagnosis of Schistosomiasis in areas of endemicity is the microscopic detection of eggs in stool and urine

samples, but this assay does not give reliable results and several measurements on different days are necessary for the precise diagnosis of Schistosomiasis **(Berhe *et al*., 2004).**

Several *Schistosoma* serodiagnostic assay for specific antibodies have been developed and such assay does not discriminate between active and old infection, and reinfection **(Maddison, 1987 and Doenhoff *et al.*, 1989).**

Detection of circulating *Schistosoma* antigens secreted by live *Schistosome* in body fluids with specific monoclonal antibodies has been shown to be promising approach to the detection of active infection and to assess the treatment efficacy and effectiveness of future vaccines **(Polman *et al.*, 1995 and El-Morshedy *et al.*, 1996).**

One of the main requirements for diagnosing schistosomiasis is the development of a more sensitive assay. Since the PCR and western blot technique are extremely sensitive and specific technique with widespread use in the diagnosis of infectious diseases, they were evaluated as diagnostic tools for detecting infection with *S. mansoni***(Kane, 2006).**

# Aim of the work

The aim of the present study is to compare the diagnostic value of immunoblot technique and PCR as diagnostic tools for schistosomiasis mansoni in experimentally infected mice and to detect which of these methods can be used in early diagnosis.

# ***Schistosoma mansoni***

## Epidemiology:

Schistosomiasis as a human disease has been recorded from 4000 years and still constitutes an important problem for public health. It is caused by blood flukes of the genus *Schistosoma*. This disease is endemic in the developing world and is most prevalent among the poorest populations. *Schistosomes* affect more than 200 million people; half of them are in Africa, while 1 billion remain at risk. It is estimated that 20 million people suffer from severe consequences of these chronic and debilitating disease. Some authors estimated that there were approximately 20,000 deaths related to schistosomiasis yearly **(Ross *et al*., 2002).**

Among the five species of S*chistosom*es that can infect humans, *Schistosoma mansoni* which infects about 83.31 million people worldwide. It is the most widespread of the human-infecting *Schistosomes* and is present in 54 countries. These countries are predominantly in South America, Caribbean and Africa including Madagascar **(Oliveira *et a*l., 2004).**

High prevalence of *S. mansoni* was observed in the Nile valley, especially in Sudan and Egypt. In Egypt, *S. mansoni* was higher in the Northern areas of

the Nile delta. In the New World, *S. mansoni* was reported in Brazil, Surinam, Venezuela and islands in Caribbean **(Gryseels, 2006).**

Although the distribution of schistosomiasis has changed over the past 50 years and there has been successful control programmes, the number of people estimated to be infected or at risk of infection remains unchanged **(Gryseels *et al.*, 2006).**

In highly disease-endemic areas, prevalence rates can exceed 50% among the local population and high rates have been reported among expatriates living in such areas and even among short-term travelers to these areas **(King, 2007).**

There are many risk factors associated with *S. mansoni* infection as host factors (male > 10 years old), environmental factors (rural areas containing canals infected with suitable snails and lack of control programs), behavioural factors (contact with water containing living cercariae and contamination of canal water with feces containing *Schistosome* eggs) and occupational factors (farmers are more exposed to infection due to irrigation by canal water containing living cercariae) **(Matthys *et al.*, 2007)**.

# Life cycle:

Studying the life cycle of *S.mansoni* is very important for planning of control programs, which allows destruction of the disease at different stages of the cycle **(Doehring, 1988).**

The transmission cycle requires contamination of surface water by excreta, specific fresh water snails as intermediate host and human water contact **(Webbe and El Hak, 1990).**

The life cycle includes sexual generation of adult in the definitive vertebrate host and asexual multiplication in a molluscan intermediate host. Adult worm lives in inferior mesenteric veins. The male carries the female in the gynaecophoric canal and migrate against the blood flow to the small venules of the portal venous system. The female produces approximately 300 lateral spined eggs daily (one egg every 4.8 minutes) **(Loverde and Chen, 1991)**

After that the eggs of parasite are emitted in the feces and into the water, the miracidium hatches out of the egg. The hatching happens in response to temperature, light and dilution of faeces with water. The miracidium searches for a suitable freshwater snail (*Biomphalaria alexandrina*, *Biomphalaria glabrata*, *Biomphalaria straminea*, *Biomphalaria tenagophila* or *Biomphalaria sudanica*) to act as an