Immunological Characterization of *Schistosoma mansoni* & *Schistosoma haematobium* Adult Antigens

Thesis

Submitted for Fulfillment of

M.SC. Degree In Parasitology

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2012

Abstract

The antigens used was crude worm antigens ELISA revealed that the sensitivity of *S.haematobium* and *S.mansoni* positive subjects was 78.57% for *S.mansoni* and 87.14% for *S.haematobium* The soluble adult worm antigens preparation was separated by gel electrophoresis to generate a reference gel used for western blotting. By software image analysis we found that there was a high degree of similarity in band pattern reveled from separation of *S.mansoni* and *S.haematobium* adult worm antigens.

Key word:

EITB , ELISA, Pathogenesis , Diagnosis of schistosomiasis , Immunodiagnostic , haemagglutination,

List of abreviations

(MAC): Membrane attack complex

(DAF): Decay accelerating factor

(ID) :Intradermal test

(CH): Cercaria hullen reaction

(COPT): Circum oval precipitin Test

(IHA): Indirect haemagglutination assay

(IFA): The immunofluorescence assay

(ELISA): Enzyme-linked immunosorbent assay

(ELISA- FDA): Fast dot-enzyme-linked immunosorbent assay

(SMP-EUSA):ELISA with sodium metaperiodate

(EITB): Enzyme-linked immunoelectro transfer blot

(MAMA): Monologous adult microsomal antigens

(HAMA): Homologous adult microsomal antigens

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σ *Acknowledgement* γ

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

I am extremely obliged to **Prof.Dr. Azza El-Adawy** *head of Parasitohgy Department, faculty of Medicine, Cairo University, for her encouragement ,support and advice .*

Sincere gratitude and deepest appreciation to my supervisor **Prof.Dr. Soheir Mostafa EL-Naggar** *Professor of Parasitology, Faculty Of Medicine ,Cairo University for her Keen, co-operation, guidance, continuous encouragement and enlightening vision from the early moment of the study till presentation of this work. Her meticulous revision of the work had helped me so much. I am proud to have her as my supervisor.*

I am truly grateful to **Prof.Dr. Waheed Mohamed Ali,** *Professor of Parasitology ,Faculty of Veterinary Medicine, 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.*

I feel very grateful to Prof.Dr. **Olfat Mahmoud El-Matrawy** *head of Parasitohgy Department, faculty of Medicine, Beni Suief University, for her suggestion of this thesis.*

Grateful as can be to **Dr. Enas Yahia Abu srea,** *Lecturer of Parasitology Faculty of Medicine, Beni Suief University, Through her patience, insight and vast knowledge who helped in Bringing this research into existence.*

Special thanks for all the staff and doctors of department of Parasitology, faculty of Medicine for their help and support during the present study.

I am extremely obliged to **Prof.Dr. Sabry Mohamed Tmmam** *Professor of virology, Faculty of Veterinary Medicine, Beni Suief University, for his encouragement, support and advice.*

I want to thank **my father, my mother , my brother , my sister, my husband , my sons** *for their never ending support and ever lasting care and help. Without their support, I would never made any achievement in my life.*

Contents

INTRODUCTION

Schistosomiasis is a chronic and debilitating disease, which is caused by small parasitic trematode worms ***(WHO, 2002)***.

Over 200 million people are affected, particularly the rural poor living in sub-Saharan Africa where more than 85 % of the current global burden is concentrated ***(Utzinger and Keiser, 2004).***

Microscopic detection of Schistosoma eggs in fecal samples *(Schistosoma mansoni)* or in urine *(Schistosoma haematobium)* remains the most widely used direct diagnostic method in endemic areas ***(WHO, 2002)***.

However ELISA has been shown to be a good alternative to egg counts for diagnosis of *Schistosoma mansoni* and *Schistosoma haematobium.* The technique was relatively cumbersome and requires an appropriately equipped laboratory ***(Polman et al., 2000).***

Antigens from virtually all stages of *Schistosoma* life cycle have been tested for immunodiagnostic potential. Some tests use more or less intact forms of the parasite.Indirect immunofluorescence on adult worm sections and extracts from these stages and fractionated derivatives have also been tested in ELISA ***(Doenhoff et al., 2004)***.

The antigens of schistosome larvae have not found favors for diagnostic purposes because of inferior sensitivity when compared with those from worm or egg antigens. A higher ratio of anti larval to anti adult antibody could be useful in discriminating between acute and chronic schistosome infections ***(Lunde and Ottesen, 1980)***.

Adult worms have the advantage of being the most abundant and easily obtained source of antigenic material. Crude extracts of worms work

well in ELISA and worm antigens generally give higher sensitivity than those from larvae ***(Maddison et al., 1985)***.

A number of modifications of the ELISA have been described .The dot-ELISA assay, was a modification of ELISA using nitrocellulose membrane as a test matrix .It was becoming widely used in simple qualitative research application to detect either antibodies or antigen ***(Attallah et al., 2005).***

AIM OF WORK

The aim of the present study is to detect the immunological characterization of *Schistosoma mansoni* and *Schistosoma haematobium* adult antigens and to diagnose patients with the acute phase of the illness or with low intensity infection in which egg output is rare whether in urine or stool.

Review of literature

Taxonomy:

Schistosomes belong to the phylum Platyhelminthes (flat worms). The schistosomes are placed in the subclass called Digenea. Digenea are characterized by an alternation of generations in their life cycle. This means that when an egg hatches it produces a larval form that eventually reproduces asexually.The offspring resulting from this asexual reproduction mature into adults that must reproduce sexually. Thus the reproduction behavior alters with each successive generation. **(Coon, 2005)**

Taxonomic classification of schistosomes is:

Kingdom	Animalia
Phylum:	Platyhelminthes (flatworms)
Class:	Trematoda (flukes)
Subclass	Digenea
Order:	Strigeida
Family:	Schistosmatidae
Subfamily	Schistosmatinae
Genus:	*Schistosoma*
Species:	*S. mansoni*
	S. japonicum
	S. haematobium
	S. indicum

Schistosoma antigens

An antigen or immunogen is any foreign substance which when introduced into animal stimulates a specific immune response in the form of antibody formation or cell mediated immunity. The antigen can react specifically with antibodies produced or sensitized T-lymphocytes induced.Most antigenic molecules are protein in nature but some are polysaccharides or lipopeptides and the antigenic molecules must be recognized as foreign (non-self) by the host since generally animals do not produce antibodies to their own protein ***(Weir, 1988).***

(I) Schistosomula Surface Antigens:

Antigens shed from schistosomula appear in the circulation before those from adult worms and egg. Thus, the host encounters and responds to schistosomulum (cercarial) antigens before other antigens ***(Hayunga et al., 1987)***.

Cercarial antigen was reported to have a diagnostic potential in acute human and immune infections ***(Hayunga et al., 1986 and Ali et al., 1989)***.

Preliminary characterization of this antigen has shown that it is a hydrophobic polypeptide of approximate molecular weight (MW) 41 kDa ***(Hayunga et al., 1986)***.

Extensive analysis of *S.mansoni* schistosomulum surface antigens recognized by immune IgG has demonstrated two distinct sets of epitopes. More than 90 % of such epitopes are carbohydrate (CHO) structures that cross react with the parasite egg antigen. A smaller

percentage is non-carbohydrate structures that do not cross react with the egg antigen ***(Dunne et al., 1987 and Ali et al., 1989)***.

Epitopes of both types have been shown to be the target of protective murine monoclonal antibodies (MAbs). Thus, they are potential targets of protective immune response.It has been shown that the anti-peptide antibodies, rather than the anti-CHO antibodies are more likely to contribute to protective immunity in man ***(Ali et al., 1989)***.

Human antibodies have been shown to immunoprecipitate schistosomula surface antigens of similar MW to those recognized by mice. This suggests that similar epitopes can be recognized by human antibodies ***(Simpson et al., 1986, Omer Ali et al., 1986 and Ali et al., 1989)***.

Immunoprecipitation of I^{125} labeled-schistosomulum surface antigens using sera from acutely and chronically infected Egyptian patients was undertaken. The major precipitated labeled antigens were of MW >200, MW 38 and 32 kDa antigens ***(Omer Ali et al., 1986)***.

However, no significant difference was observed between levels of anti-MW 38 and 32 kDa antibodies in acute or chronic sera. Antigens of MW > 200 kDa are dependant on CHO moieties, that cross react with schistosome eggs. While, antigens of MW 38 and 32 kDa are polypeptides cross reacting strongly with adult worm, but weakly if at all with the egg antigens. These findings suggest that similar epitopes are being shared between the respective life cycle stages ***(Omer-Ali et al., 1986)***.

II)Antigens of the adult worm:-

1) Somatic antigens:

In schistosomiasis the immunobiology comprises complex multifactorial processes in which many surface antigens and excreted molecules of the parasite play a role. A substantial portion of those molecules that direct the interaction with the host is glycosylated and it also appears that the major humoral immune response to schistosomes is directed to glycan epitopes on glycoconjugate antigens of different stages of the life-cycle***(Vermeer et al., 2003).***

Several investigators have isolated and characterized many of the schistosomiasis antigens in different developmental stages of the parasite that have a potential application in immunodiagnosis, treatment and prophylaxis against schistosomiasis ***(Attallah et al., 1999a)***.

2) Tegumental antigens:-

One particularly interesting aspect of the Schistosoma is its tegumental surface membrane which continuously covers the whole multicellular organism and is morphologically adapted to the intravascular habitat of the parasite. It exists in an apparently hepta-laminate form and has been shown to be antigenically and biochemically complex ***(Payares and Simpson, 1985)***.

The surface of the schistosomulum which is the stage of the parasite immediately after penetration of the host skin represents the only interface between this and parasite since the organism does not have a functional gut at this stage. Furthermore the schistosomulum surface is believed to be an important target of protective immunity ***(Geyer et al., 2005)***.