Trial for determination of antigenic relationship between *Schistosoma mansoni* and its intermediate snail host

THESIS

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Abstract

Intestinal schistosomiasis is a serious health disease, caused by *Schistosomamansoni*. *Biomphalariaalexandrina*snail is the intermediate host of *S. mansoni*in Egypt. Antigenic relationship between *S.mansoni*and *B.alexandrina*snail both infected and noninfected with *S.mansoni* wasstudied.*S.mansoni*crude antigen was prepared from adult stage of*S.mansoni*collected from experimentally infected mice. Crude antigens from both infected and non infected*B.alexandrina* snail were also prepared after dissecting each animal snail into two parts foot and visceral hump. These antigens were fractionated using Sodium dodecyl sulfate Poly acrylamide Gel Electrophoresis (SDS PAGE). Common bands were detected among adult worm antigen(AWA) and both infected and non infected*B. alexandrina* visceral hump antigens at 22,30 and 58 KDa , while a common band among AWA antigen and both infected and non infected foot antigens was detected at 40 KDa. Specific mice hyper immune serum (HIS)was prepared against each prepared antigen that was used in western blot.WB revealed that HIS of AWA is reactive to infected snailmore thannon infectedsnail. HIS of AWA identifieda common band in non infected snails both foot and visceral hump at 70 -75 KDaand identified a common band at 45 KDa in AWA and both infected and non infectedvisceral hump snail antigens.HIS of *B.alexandrina* Infected foot detected a common band at 68KDa amongboth infected and non infected foot antigens and AWA. HIS of *B. alexandrina* non infected visceral hump detecteda common band at 35 KDa among both infected and non infected visceral snail antigens and AWA.

key words :*Schistosomamansoni* ,*Biomphalariaalexandrina*,antigens, Western blot,SDS PAGE.

كلية الطب
الدراسات العليا والبحوث

محضر
اجتماع لجنة الحكم على الرسالة المقدمة من

الطبيبة/ شيماء محرز عبد العال

توطئة للحصول على درجة الماجستير / ~~الدكتوراه~~

في علم الطفيليات الطبية

تحت عنوان باللغة الإنجليزية

Trial For determination of antigenic relationship between Schistosoma mansoni and its intermediate Snail host.

باللغة العربية: محاولة لتحديد علاقة التضاد بين البلهارسيا المعوية والعوائل الوسيطة

بناء على موافقة الجامعة بتاريخ ١٠ / ٤ / ٢٠١٦ تم تشكيل لجنة الفحص والمناقشة للرسالة المذكورة أعلاه على النحو التالي :-

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٣. م.د. هدية عبد الهادي علي سيد أحمد ..... ممتحن خارجي

بعد فحص الرسالة بواسطة كل عضو منفردا وكتابة تقارير منفردة لكل منهم انعقدت اللجنة مجتمعة في يوم الأحد بتاريخ ٢٤ / ٤ / ٢٠١٦ بقسم الطفيليات مدرج المكتبة بكلية الطب – جامعة القاهرة وذلك لمناقشة الطالب في جلسة علنية في موضوع الرسالة والنتائج التي توصل إليها وكذلك الأسس العلمية التي قام عليها البحث .

قرار اللجنة : الطالبة بالرسالة قامت بعرض وشرح وافي ومكامل لموضوع الرسالة وتم الرد على استفسارات السادة أعضاء اللجنة بشأن الرسالة وتم مناقشة الطالبة في جميع نقاط الرسالة وتمت الإجابة بطريقة علمية مقنعة وافية.

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المشرف الممتحن
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الممتحن الداخلي

الممتحن الخارجي
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عصام

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List of Abbreviations

APO :	Amebocyte producing organ
AWA :	Adult worm antigen
***B. alexandrina*:**	*Biomphalaria alexandrina*
***B. glab*rata:**	*Biomphalaria glabrata*
BSA :	Bovine serum albumin
C:	Celsius
CA :	Cercarial antigen
CAA :	Circulating anodic antigen.
CCA :	Circulating cathodic antigen
DNA :	De oxy ribonucleic acid
ELISAs :	Enzyme-linked immune sorbent assays
EITB:	Enzyme linked immune transfer blot
FREP:	Fibrinogen related protein
g:	Gram
HBV :	Hepatitis B virus.
HCV :	Hepatitis C virus.
HIS :	Hyper immune serum
Hsp70:	Heat shock protein 70
Hr:	Hour
IFATs:	Immune fluorescent antibody tests
Ig:	Immunoglobulin.
IgA :	Immunoglobulin A.
IgE:	Immunoglobulin E.
IgG:	Immunoglobulin G.
IgM:	Immunoglobulin M.
IHAs:	Indirect haemagglutination assays

IDS:	Internal defense system
kDa:	Kilo Dalton.
LMW:	Low Molecular Weight
L:	liter
ml:	milliliter
Min:	Minute
MAbs *:*	Mono clonal antibodies
M:	Mole
MA:	Milli ampere
mm:	milli meter
M.W:	Molecular Weight
MWS:	Molecular Weight Standard
N.C.:	Nitrocellulose
PCR:	Polymerase chain reaction.
PBS:	Phosphate buffered saline
PEPCK:	Phosphoenol pyruvate carboxy kinase
PoMuc :	Polymorphic mucins
RNA:	Ribonucleic acid
r.p.m:	Revolution per minute
SEP :	Secretory-excretory products
SDS:	Sodium dodecyl sulfate
PAGE :	Polyacrylamide Gel Electrophoresis
SEA:	Secretory egg antigen
***S. haematobium* :**	*Schistosoma haematobium*
S .m	*Schistosoma mansoni*
***S. mansoni* :**	*Schistosoma mansoni*
S. japonicum :	*Schistosoma japonicum*
S. mekongi:	*Schistosoma mekongi*
***S.intercalatum*:**	*Schistosoma intercalatum*

SBSP:	Schistosome Biological Supply Program Unit
Spp:	Species
SWAP :	Soluble adult worm antigen preparation
V:	Volt
WHO :	World Health Organization
WB:	Western blot
4C1N:	4-chloro-1-Naphthol
µ l:	Micro liter

Introduction

Schistosomiasis is a public health problem in many developing countries, *S.mansoni* is the most widespread species of the causative trematode parasite**(WHO ,2002).**After malaria and intestinal helminthiasis, schistosomiasis is the third most devastating tropical disease in the world, being a major source of morbidity and mortality for developing countries in Africa, South America, the Caribbean, the Middle East, and Asia. ***(*WHO, 2010).**The prevalence of this parasite in human population depends on the number of infected snails in the area**.** The specificity of host parasite interactions has received great attention by parasitologists and evolutionary biologists **(Blair *et al*., 2001).** Common antigen fractions had been demonstrated to be shared between schistosomes stages miracidia, sporocysts ,cercariae and adult and their intermediate hosts **(De Santana *et al*.,1992).**

Numerous genetic and physiological factors in both the snail and the parasite are critical for determining the interaction between *S.mansoni* and *Biomphalaria*, the most important of which is the internal defense system (IDS) of the snail **(Negrão-Corrêa*et al*., 2007 and Abou El Naga *etal*., 2010)**.The parasite can escape the IDS by two mechanisms, molecular mimicry and antigenic masking. In the molecular mimicry, the parasite expresses glycoprotein epitopes on its surface that mimic host molecules. Common antigens between different species of *Schistosoma* and their intermediate hosts have been reported **(Lehr*et al* .,2008)**. These molecules could be used in the diagnosis of schistosomiasis **(*Chacónet al*., 2000)**.

The presence of common antigenic fractions between snail host tissues and Schistosomes directed many researchers to focus their work on the vaccination of the final host of *S.mansoni* with the constituents of its intermediate host (snails); these constituents include protein, nucleoprotein, lipid and carbohydrate **(Tolba *et al*., 1995)**.

Aim of the work

To demonstrate of antigenic similarity between *S.mansoni* and its intermediate snail host *B. alexandrina* using SDS PAGE and Western blot technique.

These common antigens could be used as candidates for the serodiagnosis of schistosomiasis and possible vaccination of final host of *S.mansoni*

Biomphalaria alexandrina snail

History and Taxonomic Classification:

Biomphalaria alexandrina is specific aquatic snails in a wide variety of fresh water habitat It is an air breathing snails .It serves as an intermediate host of *S.mansoni* in Egypt **(William and Hunter,1968 and Abuo El Naga, 2013).***B.alexandrina* snails are prevalent in both upper and lower Egypt, but during the last decade .It becomes the most dominant species in the Nile Delta forming a main threat for schistosomiasis transmission in the north of Egypt **(WHO, 2002).**

Taxonomy: according to **Van Damme and Ghamizi (2010)**

Kingdom:	Animalia,
Phylum:	Mollusca,
Class:	Gastropoda (cuvier1797),
Order:	Hygrophila,
Family:	Planorbidae (Rafinesque1815),
Genus:	*Biomphalaria* (preston1910),
Species:	*alexandrina* (Ehrenberg1831**)**

Morphology

1-Shell of *Biomphalaria alexandrina*

In *Biomphalaria* species, the shell is discoid, sinistral with a rounded to oval aperture. Identification of *Biomphalaria* shell needs to know several quantitative parameters including (a) shell height and diameter taken at their largest size **(Mandahl-Barth, 1957)** ;(b) whorl number(c)shape of whorls and aperture.