Evaluation of the serodiagnostic potential of tegumental antigens in human fascioliasis

Thesis submitted for partial fulfillment of Master degree in Parasitology

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Abstract:

Background: Fascioliasis was recognized as a serious public health problem in human with an estimated 17 million people infected worldwide. Enzyme Linked Immunosorbent Assay (ELISA) is considered the most sensitive and specific as an adjuvant to faecal analysis. Specificity of ELISA depended mainly on the degree of specificity and purity of the used antigen as well as history of the tested sera. Most of the immunodiagnostic assays of human fascioliasis rely on antibody detection using crude worm antigens of which the Somatic (S) and Excretory-secretory (E-S) ones constitute a major component. Also Tegumental proteins are an important source of immunodiagnostic antigens. Several *F. gigantica* antigens have been purified to enhance the specificity of the diagnostic assays from which *F. gigantica* T Ag from which the 16.5 KDa subunit was highlighted for further investigation. The different immunodiagnostic response between the different antigens and their fractions was an urge for us to study some of

their responses comparatively in a simple, ELISA technique.

Objective: The present study aims to comparatively evaluate the diagnostic performance of T Ag and its 16.5 KDa subunit prepared from *F. gigantica* adult fluke to S and E-S Ags, in a standard total indirect IgG home-made conventional ELISA for serological evaluation of human fascioliasis.

Study design: For serving this study, *F. gigantica* worms were collected from bile ducts of naturally infected cattle from a local abattoir at Cairo, Egypt, then Ags were prepared; S, E-S, T Ag and 16.5 KDa tegumental subunit (by fractionation of tegumental antigen through SDS-PAGE then elution from the gel). These Ags were introduced to an indirect TIgG ELISA. Three groups of patients were involved, Group I (15 fascioliasis patients), Group II (patients with other parasitic infections and negative for fascioliasis (8 cases of hydatidosis, 7 cases of intestinal schistosomiasis, 3 cases of amoebiasis and 2 cases of toxoplasmosis) and Group III (normal control group), it included 15 healthy individuals, confirmed to be negative for fascioliasis and the above mentioned parasitic diseases, then the results were statistically evaluated.

Results: in TIgG-ELISA, the highest sensitivity calculated from ROC test was obtained by the T Ag (100%) followed by S Ag (86.6%) followed by E-S (73.3%) and least value was obtained

from 16.5 KDa subunit (53.3%). As for the specificity, the highest value was obtained from T Ag (100%) followed by E-S Ag (79.2%) then the 16.5 KDa subunit (75%), and least value was obtained from S Ag (70.8%). The highest AUC value (1.0) was that of T Ag which was used as a reference value for the rest of the results. There was a significant outcome between the AUC values of T Ag compared to S and E-S Ags. While a highly significant outcome was found when compared to the 16.5 KDa T subunit. The dot diagram revealed that T Ag has the highest sensitivity and specificity.

Conclusions: The study highlighted the potential of *F. gigantica* T Ag over S, E-S and the 16.5 KDa T subunit in diagnosis of human fascioliasis, it seems to remain the most promising antigen as regards: sensitivity, specificity and diagnostic accuracy in conventional TIgG ELISA. This performance was followed by S and E-S Ags which showed nearly equivocal results. Unexpectedly, there was a poor diagnostic outcome from our selected 16.5 KDa T subunit.

Keywords: Fascioliasis, *F.gigantica*, ELISA, Tegumental anti gen, 16.5 KDa subunit, Somatic antigen, Excretory-secretory antigen.

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Contents

List of Abbreviations

°C : Celsius degree
µg : Microgram
µl : Microliter
Ab : Antibody
Ag : Antigen
AUC : Area under the curve
AWV : Adult worm vomit
BSA : Bovine serum albumin
CB : Cathepsin B
CIEP : Countercurrent immunoelectrophoresis
CL : Cathepsin L
CL 1 : Cathepsin L1
CT : Computed axial tomography
D. water : Distilled water
EITB : Enzyme immunoelectrotransfer blot
ELISA : Enzyme-linked immunosorbent assay
ERCP : Endoscopic retrograde cholangio-pancreatography
E-S : Excretory-Secretory
F. hepatica : *Fasciola hepatica*
F. gigantica : *Fasciola gigantica*
FABP : Fatty acid binding protein
FAST-ELISA: Falcon Assay screening test, enzyme-linked immunosorbent assay
FhAWV : *Fasciola hepatica* adult worm vomit
FhESP : *Fasciola hepatica* excretory secretory products
FhSAP2 : *F.hepatica* saposin-like protein-2
FgTAg : *F. gigantica* tegumental antigen
FhTAg : *F. hepatica* tegumental antigen

Fig : Figure
gm : gram
IFN : Interferon
Ig : Immunoglobulin
IHAT : Indirect haemagglutination test
IFA : Indirect immunofluorescence assay
IL : Interleukin

List of Abbreviations (Cont.)

KDa : Kilo Dalton
LAP : Leucine aminopeptidase
L. : Lymnaea
M : Molar
min : Minute
ml : Milliliter
mM : Milli mole
MRI : Magnetic Resonance Imaging
MW : Molecular weight
N. : Normal
NPV : Negative predictive value
O.D : Optical density
PBS : Phosphate buffer saline
PCR : Polymerase chain reaction
PMSF : Phenyl methyl sulfonyl fluoride
PPV : Positive predictive value
ROC : Receiver Operating Characteristic
rpm : revolutions per minute
rproCL1 : Recombinant procathepsin L cystein proteinase
S. mansoni : *Schistosoma mansoni*

SDS-PAGE: Sodium dodecyl sulfate polyacrylamide gel electrophoresis
Spp. : Species
TGF β : Tumor necrosis factor β
Th-1 : T helper 1
Th-2 : T helper 2
TNF α : Tumor necrosis factor alpha
TSE : Total soluble extract
WHO : World health organization
MDA : mass drug administration
T :tegumental

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Introduction

Fascioliasis was recognized as a serious public health problem in human **(WHO, 2005)** with an estimated 17 million people infected worldwide **(Mas-Coma, *et al.*, 2005)**. It is a major parasitic disease that affects the health of humans and herbivorous animals. Worldwide losses in agriculture due to fascioliasis are estimated at over 2 billion dollars per year due to an increased in animal mortality and a reduction in productivity **(Spithill *et al.*, 2009 and Torgerson and Claxton, 2009)**.

Detection of anti-fluke antibodies in serum is considered a sensitive and reliable means for diagnosing acute infections and can also be used as an adjuvant to faecal analysis for the diagnosis of latent and chronic infections **(Hillyer *et al.*, 1992).**

Immunological techniques are superior in the diagnosis of human fascioliasis to molecular techniques which are still at experimental stage and very expensive. Immunodiagnosis aims to detect worm-specific antibodies in serum samples or worm-specific antigens in serum or stool samples **(WHO, 2012)**.