

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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2016-2017

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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 antigen, 16.5 KDa subunit, Somatic antigen, Excretory-secretory antigen.



*First, thanks are all due to **Allah** for Blessing this work until it has reached its end, as a part of his generous help throughout our life.*

*My profound thanks and deep appreciation to **Prof. Dr. Iman Moawad Abdelsalam**, Professor of Parasitology, Faculty of Medicine, Ain Shams University for her great support and advice, his valuable remarks that gave me the confidence and encouragement to fulfill this work.*

*I would like also to express my deep gratitude to **Dr. Rania Mohammad Sarhan**, Assistant Professor of Parasitology, Faculty of Medicine, Ain Shams University for her generous help, guidance and patience through all stages of this work.*

*I wish to thank **Dr. Abeer Fathy Badawy**, Assistant Professor of Parasitology, Faculty of Medicine, Ain Shams University, for her continuous encouragement.*

*I am extremely sincere to **my family** who stood beside me throughout this work giving me their support.*

Ghada Hussien Mohammed

Contents

	Page
Acknowledgment	i
List of abbreviations	iv
List of figures	vi
List of tables	viii
Introduction	1
Review of Literature	6
Historical Review	6
Taxonomic Classification	7
<i>Fasciola</i> species	7
Morphology	8
Life cycle	9
Epidemiology and geographical distribution	10
Pathogenesis	12
Clinical picture	13
Prognosis and Complications	15
Imunopathogenesis	17
Diagnosis	20
Treatment	24
Medical treatment	24
Surgical treatment	26
Prevention and control	26
<i>Fasciola</i> antigens	28
Somatic (S) antigens	29
Excretory-Secretory antigens (E-S)	31
Tegumental antigens	33
Purified antigens	33
Recombinant antigens	42
Applications of different types of ELISA in diagnosis of human fascioliasis	45
Aim of the work	49

Materials and Methods	51
I Collection of worms and Preparation of Antigens	51
A.Collection of worms	51
B.Preparation of Antigens	52
a. Somatic (S) Ag	52
b. Excretory-secretory (E-S) Ag	54
c.Tegumental antigen (T Ag)	56
d.16.5 KDa tegumental subunit	59
i. Separation of TAg by Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)	60
ii. Elution of the purified FgT Ag subunit (16.5KDa) from the SDS-PAGE gel by ultrafiltration using Pall Nanosep Microcentrifugal Device (0.45 μ) (Pall Life Sciences - USA)	78
II Conventional home made Indirect TIgG Enzyme linked immunosorbent assay (ELISA)	83
A-Selection of cases and grouping of individuals included in the study	83
B-Conventional Indirect TIgG ELISA:	85
Statistical Analysis	90
Results	94
Discussion	110
Summary	121
Conclusions and recommendations	128
References	130
Arabic summary	--

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
<i>F. hepatica</i>	: <i>Fasciola hepatica</i>
<i>F. gigantica</i>	: <i>Fasciola gigantica</i>
FABP	: Fatty acid binding protein
FAST-ELISA	: Falcon Assay screening test, enzyme-linked immunosorbent assay
FhAWV	: <i>Fasciola hepatica</i> adult worm vomit
FhESP	: <i>Fasciola hepatica</i> excretory secretory products
FhSAP2	: <i>F. hepatica</i> saposin-like protein-2
FgTAg	: <i>F. gigantica</i> tegumental antigen
FhTAg	: <i>F. hepatica</i> 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
<i>S. mansoni</i>	: <i>Schistosoma mansoni</i>

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

List of Figures

Fig.	Subject	Page
1	<i>Fasciola hepatica</i> adult, <i>Fasciola gigantica</i> Adult	9
2	Life cycle of <i>Fasciola</i> spp.	10
3	Global distribution of fascioliasis.	11
4	Histological sections of acute and chronic fascioliasis.	13
5	<i>Fasciola gigantica</i> egg in an unstained wet mount (x400)	21
6	Adult <i>F. gigantica</i> worms after retrieval from bile duct of infected liver of cattle.	51
7	High Protein and Peptide Recovery Detergent Removal Resin kit step by step protocol.	59
8	Standard twin-plate mini gel unit.	62
9 A	Crude TA protein bands separated by SDS-PAGE in a 12% gel stained by comassie blue stain	72
9 B		73
10	Electrophoresis run on dual slab cell	76
11	Pall Nanosep Microcentrifugal Device	81
12	An SDS-PAGE of 70 ug/ml concentration of TA in a 12% gel.	82
13	Receiver Operating Characteristic (ROC) curve representing the plot of sensitivity versus 1-specificity for <i>Fasciola gigantica</i> Somatic Ag tested in TIgG-ELISA using <i>Fasciola</i> and non <i>Fasciola</i> sera.	95
14	Receiver Operating Characteristic (ROC) curve representing the plot of sensitivity versus 1-specificity for <i>Fasciola gigantica</i> Excretory secretory Ag tested in TIgG-ELISA using <i>Fasciola</i> and non <i>Fasciola</i> sera	98
15	Receiver Operating Characteristic (ROC) curve representing the plot of sensitivity versus 1-specificity for <i>Fasciola</i>	100

Fig.	Subject	Page
	<i>gigantica</i> Tegumental Ag tested in TIgG-ELISA using <i>Fasciola</i> and non <i>Fasciola</i> sera	
16	Receiver Operating Characteristic (ROC) curve representing the plot of sensitivity versus 1-specificity for <i>Fasciola gigantica</i> Tegumental Ag subunit (16.5 KDa) tested in TIgG-ELISA using <i>Fasciola</i> and non <i>Fasciola</i> sera	102
17	Bar chart comparing the diagnostic accuracy between all <i>Fasciola gigantica</i> prepared antigens.	106
18	ROC curves for the comparative analysis between all <i>Fasciola gigantica</i> prepared antigens (Somatic, Excretory-Secretory, Tegument and Tegumental 16.5 KDa subunit) in TIgG-ELISA.	
19	Comparative results of sensitivity and specificity obtained from TIgG- ELISA results calculated from ROC test on using the 4 antigens (Somatic, Excretory-secretory, Tegumental and the desired subunit (16.5 KDa).	107
20	Dot diagram plotting the detection of anti <i>F. gigantica</i> antibodies in sera from cases and control groups of the study.	108

List of Tables

Table	Subject	Page
1	Optimum antigen concentration, sera dilution and conjugate dilution for the prepared antigens.	94
2	Reactivity of <i>Fasciola gigantica</i> and non <i>Fasciola</i> sera against adult fasciola somatic (S) Ag in a TIgG ELISA.	95
3	Calculated parameters derived from ROC analysis of <i>Fasciola</i> Somatic Ag tested in TIgG ELISA.	96
4	Reactivity of <i>Fasciola</i> and non <i>Fasciola</i> sera against adult <i>Fasciola</i> Excretory-Secretory (E-S) Ag in a TIgG ELISA.	97
5	Calculated parameters derived from ROC analysis of <i>Fasciola</i> Excretory-Secretory(E-S) antigen tested in TIgG against <i>Fasciola</i> and non <i>Fasciola</i> sera.	99
6	Reactivity of <i>Fasciola</i> and non <i>Fasciola</i> sera against adult <i>Fasciola</i> fluke Tegumental Ag (TA) in a TIgG ELISA.	99
7	Calculated parameters derived from ROC analysis of <i>Fasciola</i> TA tested in TIgG against <i>Fasciola</i> and non <i>Fasciola</i> sera	101
8	Reactivity of <i>Fasciola</i> and non <i>Fasciola</i> sera against adult <i>Fasciola</i> TA subunit (16.5 KDa) assessed in a TIgG ELISA.	101
9	Calculated parameters derived from ROC analysis of <i>Fasciola gigantica</i> TA subunit (16.5 KDa) tested in TIgG against <i>Fasciola</i> and non <i>Fasciola</i> sera	103
10	Comparative analysis of the mean optical density of TIgG ELISA test using <i>Fasciola</i> S, E-S, TA and TA subunit 16.5 KDa between	104

Table	Subject	Page
	<i>Fasciola</i> and non <i>Fasciola</i> sera.	
11	Comparative analysis between the diagnostic accuracy, (AUC), Z and P-value of S, E-S Ag, TA and TA subunit (16.5 KDa) in TIgG-ELISA	105

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**).