بسم الله الرحمن الرحيم

"وَمَا أُوتِيتُمْ مِنَ الْعِلْمِ إِلا قَلِيلاً"

صدق الله العظيم

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Evaluation of Quantitative Immunomagnetic Bead ELISA technique Using Super-Paramagnetic Nanoparticles in Diagnosis of Schistosomiasis haematobium

Thesis

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LIST OF ABBREVIATION

APS	Ammonium persulfate
APT	Antimony potassium tartrate
As	Acute schistosomiasis
BPB	Bromo phenol blue
BSA	Bovine serum albumin
C	Complement
CAA	Circulating anodic antigen
CCA	Circulating cathodic antigen
CD	Cluster of differentiation
CD	Cleome droserifolia
CE	Cercarial elastase
CFA	Complete Freund's adjuvant
CL	Cathepsin L
CNS	Central nervous system
CSA	Circulating schistosomal antigen
DEAE	Diethylaminoethyl
DOC	Deoxy cholate
dist. H_2O	Distilled water
ECA	*Escherichia coli*
EIA	Enzyme immune assay
EITB	Enzyme linked immunosorbent assay
ELISA	Enzyme linked immunosorbent assay

EPO	Eosinophil peroxidase
E/S	Excretory secretory molecules
FAST	Falcon assay screening test
Fc	Fragment of crystallization
FCS	Fetal calf
FGS	Female genital schistosomiasis
GASP	Gut associated schistosome proteoglycan
HAMA	Haematobium adult microsomal antigen
Hb	Haemoglobin
HEPES	Hydroxyethylpiperazine- N-2-ethane-sulfonic acid
H_2O_2	Hydrogen Peroxidase
HRP	Horse radish peroxidase
HIV	Human immune deficiency virus
HPV	Human papillomavirus
IFA	Incomplete Freund's adjuvant
IFN-γ	Interferon gamma.
IHA	Indirect haemagglutination assay
IHAT	Indirect haemagglutination test
IL	Interleukin
IMB	Immunomagnetic bead
IMB-ELISA	Immunomagnetic bead ELISA
IVP	Intervenous pyelography
kDa	Kilodalton
LDNF	Lacidinac fucosylate

m	Mole
MAC	Membrane attach complex
mAb	Monoclonal antibody
MAMA	Mansoni adult microsomal antigen
MBL	Mannosel binding lectin
MIFc	Merthiolate iodine formaldehyde concentration technique
Mw	Molecular weight
NaCl	Sodium chloride
Na OH	Sodium Hydroxide
Na2 HPO4	Monobasic sodium phosphate
Na2CO3	Sodium carbonate
NaH2 PO4	Dibasic sodium phosphate
NaHCO3	Sodium bicarbonate
NPV	Negative predictive values
OD	Optical denisty
OPD	O-phenyl diamine dihydrochloride
pAb	Polyclonal antibody
PBS	Phosphate buffered saline
PBS-T	PBS-Tween
PCR	Polymerase chain reaction
PI	Post-infection
PPV	Positive predictive values
P- value	Probability value
PZQ	Praziquantel

r	Correlation coefficient
RPMI	Rosewell park memorial institute
RRU	Rabbit Research Unit
SAWA	Soluble adult worm antigen
SD	Standard deviation
SDS-PAGE	Sodium Dodecyl Sulphate –Polyacrylamide gel Electrophoresis
SEA	Soluble egg antigen
S.	*Schistosoma*
Sm13	*Schistosoma mansoni* 13 kDa tegumental antigen
SPP.	*Species*
T	Tween
$t_{1/2}$	Half life time
TBRI	Theodore Bilharz Research Institute
TEMED	Tetramethylenediamine
Th	T-helper cell
TNF-α	Tumor necrosis factor alpha
v	Volume
v/v	Volume/volume
w	Weight
wk	Week(s)
w/v	Weight/volume
WHO	World Health Organization.

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ABSTRACT

Schistosomiasis haematobium is a serious public health problem in Egypt. Detection of *S. haematobium* antigens is a better immunodiagnostic tool than determination of the antibody level. We developed a novel immunomagnetic bead ELISA based on IgG for detection of CL antigen in sera and urine of patients infected with *S. haematobium*.

Detection of CL Ag in serum using IMB-ELISA gave a sensitivity of 95%, a specificity of 93.3% compared to other parasitic infections group and 100% compared to healthy control group. While, detecting the same Ag in serum using sandwich ELISA gave a sensitivity of 85%, a specificity of 88.3% compared to other parasitic infections group and 100% compared to healthy control group.

On the other hand, Detection of CL Ag in urine using IMB-ELISA gave a sensitivity of 91.6%, a specificity of 93.4 % compared to other parasitic infections group and 100% compared to healthy control group. While, detecting the same Ag in urine using sandwich ELISA gave a sensitivity of 83.3 %, a specificity of 81.8 % compared to other parasitic infections group and 100% compared to healthy control group.

The novel assay appears to be a sufficiently sensitive and feasible assay for detection of schistosomal antigenemia and the evaluation of its potential use in human schistosomiasis is in progress.

Key words: Cathepsin L antigen (CL); Schistosomiasis; *Schistosoma haematobium (S. haematobium)*; Immunomagnetic bead ELISA technique (IMB-ELISA).

INTRODUCTION

AND

AIM OF WORK

Human schistosomiasis is a chronic, debilitating parasitic disease and is caused mainly by three species of the genus *Schistosoma (S.): S. haematobium, S. japonicum* and *S. mansoni* (**He *et al.*, 2005**). More than 600 million people are at risk with about 200 million actually infected in 74 countries mainly in the tropics and subtropics (**Ruelas *et al.*, 2006**). *Schistosomiasis haematobium* is an important public health problem in Africa and the Middle East affecting more than 110 million people in rural, agricultural and peri-urban areas (**WHO, 2008**). Schistosomiasis is second only to malaria in terms of public health importance (**Abdulla et al., 2007**). It is associated with a variety of clinical syndromes that may lead to severe morbidity (**Bahgat et al., 2010**). The ancient Egyptians contracted the disease more than 4000 years ago (**El-Zayadi, 2004**).

Adult worm pairs of *S. haematobium* are found in copula within venous plexuses surrounding the bladder and ureters. Hundreds of eggs are laid by each female worm per day, and these gradually find their way into the lumen of the bladder (**Blanchard, 2004**). The disease is characterized by painful micturition, dysuria, hematuria, proteinuria and the presence of schistosome eggs in the urine of infected persons (**Bosompema et al., 1996; Conor et al., 2002**). In the later stages immune-mediated granulomatous response to parasite eggs lead to granuloma formation in the lower urinary tract; which is the main cause of the pathology in the bladder (**Pearce and McDonald, 2002).** Schistosomiasis is associated with debilitating morbidity manifested by sequelae such as iron deficiency anemia, cognitive impairment, lassitude, stunting growth (**Savioli et al., 2004**) and predisposition to cancer of the bladder especially in adults (**Michaud, 2007**).

Routine diagnosis of *Schistosoma haematobium* infections can be done by detection of eggs in urine samples where eggs can be