Evaluation of Cystatin-Capture and Periodate-Treated ELISA in Serodiagnosis of Toxoplasmosis and Hydatidosis

A thesis submitted to Faculty of Medicine – Ain Shams University for partial fulfillment of Doctor Degree in Parasitology

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Protocol

Introduction

Cysteine proteinases (proteases) are proteolytic enzymes found in the excretory-secretory products of different parasitic helminthes **(Tort et al., 1999)** and protozoa **(Rosenthal, 1999).** Purification of cysteine proteinases is a complex and time consuming process that can be substituted by the use of cystatin a protein inhibitor of cysteine proteinases which forms tight binding with these proteinases making them accessible to serum antibodies without the need for purified enzymes **(Bode et al., 1988).** Cystatin, present in chicken egg white, is a protein inhibitor of cystiene proteinases with a very low dissociation constant value and thereby, has a high specificity for them **(Anastasi et al., 1983).** The cystatin molecule forms a wedge shaped edge complementary to an active site cleft of a cysteine proteinase, and the edge penetrates into the cleft forming a tight binding between cystatin and cysteine proteinases **(Bode et al., 1988).** The high specificity of cystatin to proteinases made it a suitable capture-reagent for detecting anti-cysteine proteinases**.** Cystatin has been used successfully as a capture agent in ELISA to detect cysteine proteinas antibodies without the need for purified proteinases for the diagnosis of Chagas disease **(Scharfstein et al., 1995),** human fascioliasis **(Tawfeek and Hussein, 2000)**, schistosomiasis *mansoni* **(Chappell et al., 1990),** trichomoniasis **(Tawfeek et al., 2003)** and experimental trichinellosis **(Mahmoud and Mostafa, 2003).**

Toxoplasma gondii is an obligate intracellular parasite that can invade and replicate within any nucleated cell of vertebrate hosts, including humans **(McAuley et al., 1994).** As an opportunistic human pathogen, *T. gondii* causes devastating disease in immunocompromised individuals, especially AIDS patients and congenitally infected neonates. *Toxoplasma* encephalitis is the most common cause of central nervous system infection in patients with AIDS and is uniformly fatal unless it is treated **(Black and Boothroyd, 2000).**

As one of *Toxoplasma gondii* produced cystiene proteinases, it was found that cathepsin B, toxopain-1, localizes to the unique rhoptry organelle of *T. gondii*, which is required for parasite invasion. One crucial biological function of toxopain-1 appears to be the processing of rhoptry proteins **(Que et al, 2002, Sajid and McKerrow, 2002).** Toxopain-1 is also secreted in the parasitophorous vacuole, where it may be involved in the degradation of host cell peptides **(Sijwali et al., 2001).** Inhibiting the expression of toxopain-1-specific mRNA and protein significantly decreased the capacity of the parasites to multiply and invade in vitro **(Que et al., 2004).** To date, only one cathepsin B, one cathepsin L, one cathepsin D, and three cathepsin Cs have been detected in the *Toxoplasma* genome project **(Que et al., 2007).**

Echinococcus granulosus is the causative agent of cystic hydatid disease or hydatidosis, a disease of a global distribution. Though the liver is the most frequently involved site, the cysts can develop in almost all organs of the body. Main clinical symptoms in humans include liver dysfunction, lung problems, ascites, abdominal pain,

hepatomegaly, splenomegaly and central nervous system disorders. Because there is not any production of the parasite into faeces, the laboratory diagnosis of hydatidosis mainly rests upon the detection of anti-hydatid antibodies in serum samples as well as clinical and radiological data **(Markell and Voge, 1999).**

The cysteine proteinase cathepsin K was purified by anion exchange and gel filtration and its identity was confirmed by Western blotting with a cathepsin K-specific antibody. Cathepsin K was then immunolocalized to the hydatid cyst wall sections from infected hosts **(Diaz et al., 2000).**

Immunodiagnostic methods such as latex agglutination, indirect haemagglutination, complement fixation, indirect fluorescent antibody, precipitation tests, western blotting and ELISA tests, have been used in many laboratories and could confirm the diagnosis of hydatidosis **(Biava et al., 2001)** and toxoplasmosis **(Black and Boothroyd, 2000).** The sensitivity of these assays varies from 60% in immunoelectrophoresis up to 90% in indirect haemagglutination and ELISA **(Wilson and Schantz, 1991).**

Reduced sensitivity may be partially due to the presence of carbohydrate molecules **(March et al., 1991).** Glycosylated epitopes have an important role in cross-reaction between different parasites. Oxidation with sodium periodate is useful in the structural analysis of glycosylated molecules and has been used to elucidate the role of the carbohydrate portion of glycoproteins in the reactivity by serology **(Hamburger et al., 1982; Schallig and van Leeuwen 1996).** ELISA performed with sodium

periodate increased sensitivity in serodiagnosis of hydatid disease **(Sterla et al., 1997).** When sodium periodate was used in ELISA as screening test for schistosomiasis, the specificity improves from 73 to 97%, reducing the number of false positives and the sensitivity was 99%. Sodium periodate increased the specificity of ELISA , reduced cross-reactivity with serum samples from cases infected with other parasites, maintained its high sensitivity and improved its value in evaluating therapeutic efficacy **(Alarco´n de Noya et al., 2000,Haung et al., 2003).**

Saving time, work forces, and reaching to a valuable diagnosis result as soon as possible, are the factors that necessitate improving a serological test like ELISA to be used in different laboratories **(Rokni, et al., 2006).**

Aim of the work

The aim of this work is to evaluate the use of cystatin-capture and periodate-treated ELISA tests for diagnosis of toxoplasmosis and hydatid disease in comparison to the commercially available immunodiagnostic tests.

Plan of the work

1. Collection of serum samples of patients of:

    - Toxoplasmosis (**30 cases** confirmed by clinical data and Sabin Feldman test).

    - Hydatidosis (**30 cases** confirmed by clinical data and radiology).

    - Other parasitic disease (**10 samples**).

- In addition to sera of normal healthy individuals as control samples (**10 samples**).

[All samples will be collected from patients attending Diagnostic and Research Unit in the parasitology Department - Faculty of Medicine - Ain Shams University].

2. In vivo maintenance of *Toxoplasma gondii* RH strain.

3. Preparation of *Toxoplasma gondii* crude antigen.

4. Preparation of hydatid cyst fluid antigen.

5. Performance of IHAT for each disease.

6. Performance of basic ELISA for each disease.

7. Performance of cystatin-capture ELISA for each disease.

8. Performance of modified ELISA treated with sodium periodate for each disease.

9. Evaluation of specificity and sensitivity of each type of ELISA for each disease by testing heterologus group of sera of patients infected by other parasitic disease and sera of normal individuals.

10. Evaluation of the results of cystatin-capture and periodate-treated assays in comparison to the results of commercially available test for each disease (IHAT and basic ELISA).

Location of the work

(All steps of the work will be performed at Diagnostic and Research Unit – Parasitology Department – Faculty of Medicine – Ain Shams University).

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Content

List of Abbreviations

AE	Alveolar Echinococcosis
Ag	Antigen
AIDS	Acquired immunodeficiency syndrome
AUC	Area under curve
CCIEP	Counter current immunoelectrophoresis
CD	Clusters of differentiation
c-DNA	Cloned-DNA
CE	Cystic echinococcosis
CHD	Cystic hydatid disease
CIC	Circulating immune complexes
CT	Computed tomography
DNA	Deoxyribonucleic acid
EgAP	*Echinococcus granulosus* alkaline phosphatase
EITB	Enzyme linked immunotransfere blot
ELISA	Enzyme-linked Immunosorbent Assay
ESA	Excretory secretory antigen
FNAB	Fine needle aspiration biopsy
GRA	Granule antigen
HA-DIA	Hydatid antigen-dot immunobinding assay
HCF	Hydatid cyst fluid
HIV	Human immunodeficiency virus
IB	Immunoblotting
IEP	Immunoelectrophoresis
IFAT	Indirect florescent antibody test
IFN	Interferon
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IHAT	Indirect haemagglutination test
IL	Interleukin