Identification Of A 38 KDa Antigen In The Urine Of Schistosoma Mansoni Chronically Infected Patients Using A Specific Antibody Microeluted From A Nitrocellulose Membrane; A Diagnostic Tool

Mohamad Ali Saber[1], Mahmoud Romeih[1*] and Samir S. Mahgoub[2]
Departments of Biochemistry and Molecular Biology, Theodor Bilharz Research Institute[1] and Faculty of Medicine, Al Minia University,**

*** Present address: Department of Biochemistry and Molecular Biology, Michigan State University; Department of Science, Lansing Community College, Michigan, USA**

ABSTRACT

Since schistosomiasis is a chronic debilitating disease affecting 200 -300 million throughout the world, a major focus of research has been done to identify and characterize antigens that may have vaccine and / or diagnostic potential. In the present study, NP-40 extracted surface proteins of ***Schistosoma mansoni*** (***S. mansoni***) adult worms were subjected to 12.5% Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS-PAGE) and electrotransfered (in a Western blot experiment) onto a nitrocellulose membrane (PVDF), then, incubated with pooled sera collected from ***S. mansoni*** infected patients, sera's antibodies identified a number of NP- 40 extracted surface proteins. One of these proteins of 38 kDa molecular weight with high immunogenicity was selected. The strip of nitrocellulose membrane containing the complex of the identified protein and its specific antibody was cut guided by the molecular weight marker, then, the antibody was micro-eluted. Proteins of urine samples from the same patients were precipitated and purified over G-Sephadex column. The purified proteins of urine samples and proteins of sera were subjected to another SDS- PAGE and electro-transferred onto PVDF membrane and the micro-eluted antibody was applied in another Western blot experiment. The specific antibody identified the antigen of 38 kDa molecular weight in sera of infected patients as well as urine. The 38 kDa antigen is excreted in the urine of schistosomiasis infected patients in a stable form and was detected by a specific monoclonal antibody. Therefore, the active epitope of 38 kDa antigen could be a promising immunochemical probe for S. ***mansoni*** infection diagnosis. Further studies will be done to characterize that antigen as well as its potential as an immuno-diagnostic reagent.

Key Words: Schistosomiasis, Immuno-diagnostic, 38kDa, *S. mansoni*

INTRODUCTION:

Schistosomiasis mansoni is a chronic disease caused by infection with helminthes of genus ***S. mansoni*** (Pearce***, 2005)***.The purification, identification and characterization of schistosome antigens are needed as a step toward the diagnosis of human schistosomiasis and the development of a vaccine against the parasite. The circulating antigens of ***S. mansoni*** released from the surface of the adult worms, secretory excretory or gut associated antigens are released directly into the host blood stream (Nash ***and Deelder, 1985)***. Satisfactory diagnostic techniques are

prerequisite for the success of schistosomiasis control programs in endemic areas. Parasitological methods lack sensitivity; serological tests have been suggested to be incorporated for schistosomiasis diagnosis. Improvement of the immunodiagnostic tools depends upon the production of specific and sensitive antigens in sufficient amount to provide low cost assay. As already applied to different infectious diseases, some recombinant proteins and synthetic peptides might be used as immunogenic and specific antigens for diagnostic purposes in schistosomiasis (Luiz ***et al***., ***2007)***. Several investigators had identified and characterized many of these antigens in the urine of ***S. mansoni*** infected patients using monoclonal antibodies that have a potential application in immunodiagnosis ***(Abdeen et al., 1999 and Attallah et al., 1999)***.

The aim of the recurrent work was the identification of a secretory excretory protein fraction of ***S. mansoni*** adult worms with high immunogenicity in the urine of chronically infected schistosomiasis patients using a specific monoclonal antibody micro-eluted from a nitrocellulose membrane against one of the adult worms surface proteins with high immunogenic potential.

MATERIALS AND METHODS:

Nonidet P-40 (NP-40) soluble surface proteins extract of adult worms of *S. mansoni*.

Adult worms were mixed with NP-40 buffer containing 20 mM Tris, pH 7.4, 0.1% NP -40 and 2 mM phenyl methyl sulfonyl fluoride (PMSF), then, vortexed every 5 min for 1 hr. The pellet was spun down. The supernatant was collected and stored at -20^{o}C (Basch***, 1981***).

Sera and urine:

The samples were obtained from chronically infected patients with ***S. mansoni*** from Tropical Medicine Outpatient Clinic, Zagazig University Hospitals.

Preparation of proteins of urine:

Proteins are precipitated with fully saturated solution of ammonium sulfate, then, purified using G- sepharose column. The elute from column was dialyzed against 2 liters of Phosphate–Buffered Saline (PBS) (0.4 g NaCl, 1.44 g Na_2HPO_4 and 0.24 g KH_2PO_4 / liter) ***(Lee et al ., 1995***).

SDS–Polyacrylamide Gel Electrophoresis (SDS–PAGE) (Sambrook ***et al., 1989)***.

The resolving gel (12%) and stacking gel (5%), the gel was left 30 min for polymerization and the samples of sera and urine (10 µl each) was mixed with an equal volume of 2 X SDS gel loading buffer { 1.25 ml 1 M Tris, pH 6.8, 4.0 ml 10% SDS, 2.0 ml glycerol, 1.0 ml β–mercaptoethanol, 20 mg bromophenol blue and deionized distilled water(dd H_2O) up to 10 ml }, the samples were boiled for 5 min , then, loaded onto the gel. Electrophoresis was carried with constant current of 20 – 25 m Amp until the dye arrived at the gel front. The gel was stained in Commassie stain with rocking for 1-2 hrs, then, destained till acceptable back ground was obtained and left to dry.

Western blot analysis (Towbin ***et al., 1979)***:

Afler electrophoresis, the gel was placed in a sandwich form with a piece of PVDF membrane in a western transfer buffer (3.02 g Tris-base, 18.8 g glycine, 200 ml ethanol and dd H_2O up to a liter) at 75 -100 volts for 1 hr . The membrane was blocked in 5% non fat dry milk in PBS, washed 4 times in PBS, then, incubated with the microeluted antibody for 3 hrs at room temperature. The secondary antibody (Anti–primary antibody conj-ugated with alkaline phosphatase) was incubated with the membrane, then, washed 4 times in PBS. The substrate color reagent (Vector Laboratories) dissolved in o.1 M Tris- HCl, pH 9.5 and incubated with the membrane until good signals were developed, then, left for air dry.

Elution of antibodies from nitrocellulose strips (Beall ***and Mitchell***, ***1986)***.

The band of the selected protein after transfer from gel to PVDF membrane was cut guided by the molecular weight marker. The strip was washed 3 times in TN 20 min each (10 m M Tris- HCl , pH 8.0 , 0.15 M NaCl), then, in 0.1 M boric acid and lastly in PBS. For elution of antibody, the membrane was incubated in 0.1 M glycine, 0.15 M NaCl, pH 2.6, then, its pH was adjusted to pH 8.0 by 2 M Tris-HCl, pH 8.0 and frozen at – 70^{o}C.

RESULTS.

Detection of *S. mansoni* high immunogenic surface proteins using sera obtained from *S. mansoni* infected patients:

Sera containing antibodies identified some antigenic epitopes of the surface proteins of ***S. mansoni*** adult worms by SDS–PAGE and western blotting techniques. NP– 40 extracted surface proteins of ***S. mansoni*** adult worms were separated by SDS – PAGE, then, subjected to electro-transfer onto PVDF membrane which was probed after blocking the non-specific binding sites with sera collected from chronically infected patients with ***S. mansoni***. Antibodies identified some antigens and some of them showed high immunogenicity with different molecular weights. One of them of 38 kDa was selected to be studied. The molecular weight marker was indicated on the side of PVDF membrane, the arrow points at the identified protein **(Fig .1).**

Detection of *S. mansoni* antigen in the urine of *S. mansoni* infected patients:

The antigenic epitope reactive with the specific monoclonal antibody micro-eluted from the nitrocellulose membrane was identified in the urine samples obtained from ***S. mansoni*** infected patients by 12.5% SDS–PAGE and western blot technique, urine samples from normal persons not infected with ***S. mansoni*** were used as negative control. The micro-eluted antibody detected a protein antigen with apparent molecular weight at 38 kDa in the urine sample of ***S. mansoni*** infected patients and in sera of the same patients (as a positive control). The antibody did not react with proteins of urine samples from the non-infected individuals, also, it did not react with proteins of ***E. Coli*** lysate (both were used as negative control). **(Fig. 2)**

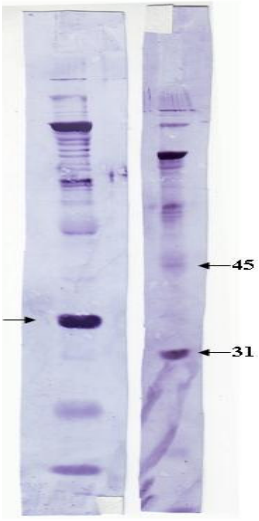

Fig . (1) Immunoblot of NP – 40 extracted surface proteins of *S. mansoni* adult worms probed with sera obtained from ***S. mansoni*** chronically infected patients. The selected protein having 38 kDa molecular weight was pointed at by an arrow.

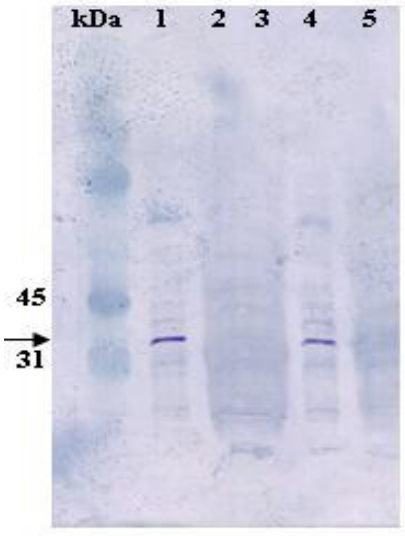

Fig . (2). Immunoblot of proteins of sera of ***S. mansoni*** chronically infected patients in lane 1 the precipitated and purified proteins of urine of the same patients in lane 4, while lanes 3 and 4 are sera proteins and the precipitated and purified proteins of urine of non infected individuals with ***S. mansoni***, ***E. Coli*** lysate (lane 5) as negative control. The molecular weight marker is indicated on the side of the nitrocellulose membrane and the arrow points at the identified protein of 38 kDa molecular weight by the micro-eluted monoclonal antibody.

DISCUSSION.

Schistosomiasis is a global health problem caused by several species of schistosome blood flukes. It is endemic in 74 developing countries, 200 million people are infected worldwide (***Ciselle et al., 2005***), causing an estimated 200.000 deaths / year ***(Chitsulo et al., 2004).*** Thus schistosomiasis has earned a category II disease ranking next to malaria for importance as a target tropical disease by World Health Organization Special Programme for Research and Training in Tropical Disease ***(WHO, 2002***).

Diagnosis of ***S. mansoni*** is based on clinical data associated with eggs detection in stool, urine and / or rectal and bladder biopsy specimens, but most of the patients of ***S. mansoni*** do not excrete eggs or excrete only few eggs in an uneven fashion, also some patients are asymptomatic or have no specific biological or clinical signs (***Ye et al ., 1998 and Valli et al., 1999***). So, diagnosis of schistosomiasis by detection of specific antibodies is likely to be more sensitive than diagnosis by traditional parasitological techniques, where circulating antigen assays can be used not only for diagnosis but also, for estimating intensity of infection (***Polman et al., 2002***).

Many antibody techniques have been developed for ***S. mansoni*** diagnosis as indirect immune-fluorescence, enzyme linked-immunosorbent assay(**ELISA**) and indirect hemagglutination. ***Doenhoff et al., 2004,*** suggested that people who are parasitologically negative and /or circulating antigen negative cannot be assumed non infected and positive serological test results do not necessarily prove an infection due to imperfect techniques and cross reactions may resulting in false positive results, for example the demonstration of eggs in urine and feces directly indicates the presence of worms but the disadvantage of this technique is the high fluctuation of egg counts. The immunological methods such as ELISA required more advanced laboratory setting but may yield a higher sensitivity particularly for antibody detection but treatment efficacy remains difficult to determine since specific antibodies continue to present long after the worms have disappeared, so, detection of parasite antigens as circulating cathodic and anodic antigens (CCA and CAA) by ELISA shows many advantages, such as demonstration of active infections or treatment effect and has a high specificity (***Van lieshout et al., 2000***). The sensitivity, specificity, positive and negative predictive values of a reagent strip test for the diagnosis of ***Schistosoma mansoni*** by detecting circulating cathodic antigen (CCA) in urine were evaluated using 184 stool and urine samples collected from schoolchildren living in relatively low endemic area of schistosomiasis mansoni living in Ethiopia (***Legesse and Erko, 2008***).

Immunodiagnostic methods based on detection of specific antibodies continue to be the most effective and practical methods for diagnosis of schistosomiasis and correlated clinically

with intensity of infection morbidity and post–treatment monitoring (***Hassan et al ., 1999***), their sensitivities lie between 65 and 95% according to different studies (***Van lieshout et al.,1992 and Pardo et al., 2004***).
Few serological tests have high sensitivity and excellent specificity for schistosoma diagnosis (***Van Gool et al ., 2002***). Western blot analysis is an interesting tool for the detection and confirmation of schistosomiasis, the ease of use, good sensitivity and specificity makes western blot useful test for diagnosis of the disease (***Annie et al., 2005***). Some investigators reported the usefulness of western blot analysis for differentiating between recent and chronic schistosoma infection (***Valli et al ., 1999***) and different ***schistosoma*** species.

The technique of western blot could be used worldwide for analysis made with crude ***S. mansoni*** antigens which is the most widely used for diagnosis of schistosomiasis (***Al–sherbiny et al., 1999***). The fundamental advantage of using complex antigens from the adult worms is due to their high yield of antigenic material (***Hamilton et al.,1998***).

In the present study, the microeluted specific antibody recognized a single 38 kDa antigen excreted in the urine of ***S. mansoni*** infected patients as determined by western blotting. The development of monoclonal antibody had led to characterization of several diagnostic and protective antigens. An antigen of 28 kDa was purified by ***Balloul et al., 1985***. ***Tarrab- Hazdai et al., 1985***, has purified 45 kDa and 30 kDa glycoprotein antigens from ***S. mansoni*** soluble cercarial extract .

Abdeen ***et al., 1999***, identified a circulating cathodic antigen at 41/42 kDa in the urine of ***S. mansoni*** infected patients using monoclonal antibody. ***Attallah et al., 1998*** identified a 74 kDa antigen in three developmental stages of ***S. mansoni*** and in the urine of ***S. mansoni*** infected patients using anti – ***S . mansoni*** monoclonal antibody (mAb).

The detection of specific antigens allow species diagnosis , 30 kDa band detection is highly specific for ***S. mansoni*** infection and the detection of 23 kDa band is specific for ***S. hematobium*** infection (***Al – Sherbiny et al., 1999)***. Since the 38 kDa antigen identified in the present study could be excreted in the urine of ***S. mansoni*** infected patients so, it could be used as immunodiagnostic reagent for schistosomiasis .

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