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# Importance of *Schistosoma mansoni* Thioredoxin Glutathione Reductase: 1- Evaluation of its Role in Schistosomiasis Diagnosis in Human

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*Schistosoma* parasite continued to be the focus of solicitude investigators to control the prevalence of the parasite. Efforts are needed for sensitive and accurate diagnosis that can be utilized to rapidly map the prevalence of the disease. The study aimed to benefit from the necessity of the *S. mansoni* for the enzyme Thioredoxin/Glutathione Reductase (TGR), in utilization of the enzyme as target antigen and to evaluate its efficacy in the diagnosis of *S. mansoni* infection patients. For reach the our goal, affinity chromatographic method has been utilized for isolation of TGR antigen from *S. mansoni* adult worm and employ this antigen for production of pAb for schistosomiasis by immunized rabbits. The pAb used in the efficacy evaluation of the purified pAb of TGR antigen in the diagnosis of *S. mansoni* in serum and urine of infected individual by indirect ELISA. The study, introduced a high diagnostic efficiency for the sandwich ELISA for detection of SmTGR antigen when applied to individuals with low or high worm burden in both serum and urine (89.73 and 87.02%, respectively). Sandwich ELISA revealed that this antigen can be relied on as a diagnostic target antigen in serum and urine.

**Key words:** *S. mansoni* diagnosis, sandwich ELISA, thioredoxin/glutathione reductase

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## INTRODUCTION

Schistosomiasis is still considered the most important public health problem in tropical and sub-tropical countries; it was considered the second major parasitic disease in the world after malaria, as it is responsible for about 500,000 deaths per year (Farias *et al*., 2010; Collins *et al*., 2011). The use of effective drugs against the parasite was not successful in reducing the number of infected cases (Corstjens *et al*., 2008). The *Schistosama* (S.) parasites imply a protective mode of action which helps it to survive for decades in the human host. This is done by using a unique set of antioxidant enzymes that inhibit by degradation of the reactive oxygen produced by the host's innate response (Simeonov *et al*., 2008). TGR and Peroxiredoxin (PrX) are the two most important enzymes in this defense mechanism. Thus, they could be considered as targets for anti-schistosomiasis drug development. The Ribonucleic Acid (RNA) silencing experiment shows that TGR is essential for parasite survival and the biochemical analysis indicate that TGR and its mammalian. It was found that a unique multifuctional enzyme, TGR replaced the absence of specialized TrxR and Glutathione Reductase (GR) enzymes in *S. mansoni*. TGR protects adult worms by making antioxidants against the hosts, thus TGR could serve as a target for anti-parasitic drugs (Alger and Williams, 2002; Maggioli *et al*., 2004; Rai *et al*., 2009).

Control strategies of schistosomiasis necessitates rapid and accurate diagnosis (Peng *et al*., 2008). On the other hand, detection of circulating antigens has been shown to be a sensitive and specific alternative for parasitology and antibody-detection methods in diagnosis of active parasitic infection (De Jonge *et al*., 1988; Van Lieshout *et al*., 1995).

As TGR enzyme consider the essential protein for survival of *S. mansoni* parasite under oxidative stress produced by the host's innate immune and the parasite inherence to secrete TGR during different life cycle stages, the study attempt to utilization of the enzyme as an antigen target and evaluate its efficacy in the diagnosis of *S. mansoni* infection patients.

## MATERIALS AND METHODS

**Experimental animals:** New Zealand white male rabbits, weighing approximately 1.5 kg that are about 3 months of age, were purchased from Rabbit Research Unit (RRU) Agriculture Faculty, Cairo University. And laboratory bred male, Swiss albino mice strain, each weighing 18-20 g, were used in this study. The animal experiments were carried out according to the internationally valid guidelines and in an institution responsible for animal ethics [Schistosome Biological Supply program Unit at Theodor Bilharz Research Institute (SBSP/TBRI), Giza, Egypt] (Nessim and Demerdash, 2000).

**Cercariae and infection:** *S. mansoni* cercariae were provided by SBSP/TBRI. Infection was performed by subcutaneous injection (s.c.) of 100 *S. mansoni* cercariae per mouse (Stirewalt and Dorsery, 1974).

**Collection of human samples:** This study was conducted on 130 *S. mansoni* infected patients from out patients' clinic and hospital at TBRI finding. Patients were chosen from those admitted at TBRI proved to have *S. mansoni* infection by routine parasitological stool examination, radiological finding of bilharziasis, cystoscopic findings of bilharzial lesions. In addition, 55 patients infected with other parasites (*Fasciola*, *Ancylostoma*, hydatid and ascarise). In addition, 50 individuals of the medical staff at TBRI served as parasite free-healthy negative control. Stool and blood samples were collected from all cases and sera were separated, aliquoted and kept at 70°C until used. Parasitological examination performed by direct smear examination and Merthiolate Iodine Formaldehyde Concentration method (MIFC) according to Jayewardene (1957), the egg count using kato-katz technique was done according to Engels *et al*. (1997).

**Parasite antigen preparation:** Mature *S. mansoni* worms were recovered by porto-mesenteric perfusion of livers of *S. mansoni* infected mice at 8-12 wk Post-Infection (PI). Deoxycholic Acid (DOC) extracted material was collected from adult worms and the supernatant was stored at -80°C until use (Maggioli *et al*., 2004). Protein content of the prepared antigens was measured by the Bio Rad Protein assay kit (Bradford, 1976).

**Purification of *S. mansoni* TGR antigen:** TGR was purified to homogeneity according to the method described by Rigobello *et al*. (1998), with some modifications. One hundred and eighty milliliters of DOC-extracted material (30 mg total protein) was submitted to ammonium sulfate fractionation according to Nowotny (1979) and Harlow and Lane (1988). Further purification of TGR antigen performed by 2'5' ADP Sepharose 4B column chromatography (Hunt *et al*., 1983; Healthcare and Bio-Sciences, 2005). The purity of the produced protein was assayed by SDS-PAGE under reducing and non-reducing conditions (Harlow and Lane, 1988; Myers, 1995).

**Assessment of reactivity of *S. mansoni* TGR by indirect ELISA:** The antigenicity of the purified target antigen was tested by indirect ELISA, for sera of *S. mansoni* and other parasites infected-human e.g., *F. gigantica*, hydatid and hookworm. This method was a modification of the original method of Engvall and Perlman (1971).

**Production of polyclonal antibodies (pAb)**

**Immunization of rabbits:** Rabbit anti-*S. mansoni* TGR polyclonal antibodies (pAb) was obtained by immunizing New Zealand white rabbits with *S. mansoni* TGR antigen. Each rabbit was injected intramusculary (i.m.) by 1 mg of *S. mansoni* TGR antigen. The rabbits received priming dose as injection at four sites [1 mg *S. mansoni* TGR antigen mixed 1:1 in Freund's Complete Adjuvant (FCA) (Sigma)]. Two booster doses were given, each was 0.5 mg antigen emulsified in Freund's Incomplete Adjuvant (FIA). The first boosting was 2 week after priming dose. The following boosting dose was given at weekly intervals.

The rabbits were bled for collection of sera 1 week later after a preliminary testing of titer by indirect ELISA. Rabbit sera that contain anti-*S. mansoni* TGR pAb was fractionated and kept at -20°C.

**Purification of rabbit anti-*S. mansoni* TGR polyclonal antibodies:** Rabbit IgG purification was based on sequential use of ammonium sulfate precipitation method (Nowotny, 1979), caprylic acid purification method (Mckinney and Parkinson, 1987) and DEAE-Sephadex A-50 ion exchange chromatography method (Sheehan and FitzGerald, 1996). After purification, the protein content was estimated by Bio-Rad protein assay (Bradford, 1976) and the purity of IgG was identified by SDS-PAGE (Laemmli, 1970). Anti-TGR IgG were conjugated with Horseradish Peroxidase (HRP) using periodate method according to Tijssen and Kurstak (1984).

**Application of sandwich ELISA for detection of SmTGR antigen in human sera and urine samples:** Sandwich ELISA based on the original method of Engvall and Perlman (1971) was used with the microplate modification of Voller *et al.* (1974), Engvall (1980), Nilsson (1990) and Venkatesan and Wakelin (1993). The antigen level was measured as Optical Density reading (O.D) at 492 nm. The results were expressed as the mean (X) O.D±SD. The O.D values equal to or less than cut off value were considered negative while those readings greater than the cut off value were considered positive (Nagy *et al.*, 2010).

**Key features in reliability of test results:** Sensitivity, specificity, Positive Predictive Values (PPV), Negative Predictive Values (NPV) and efficiency of sandwich ELISA were used for detection of *S. mansoni* antigens in serum and urine samples of *S. mansoni* infected patients calculated according to equations used by Nagy *et al.* (2010) and Allam *et al.* (2012).

**Statistical analysis:** The data are presented as mean±standard deviation of mean (X±SD). The mean values of each group were calculated from the mean values of individual patients. The mean groups were compared by one-way Analysis of Variance (ANOVA) (Campbell 1989; Snedecor and Cochran, 1989). The comparison between various groups was done using either Student's T test. Correlation between the OD of ELISA technique and the number of eggs in stool samples of infected group was performed by application of correlation coefficient (r) according to Snedecor and Cochran (1989). The data were considered significant if P values were equal to or less than 0.05 statistical analysis was performed with the aid of the Statistical Package for Social Sciences (SPSS) software computer program (Al-Mujaini *et al.*, 2011).

## RESULTS

**Purification of TGR antigen from DOC extracts of adult *S. mansoni* worms:** The DOC extracts obtained from adult *S. mansoni* worms with total protein 8 mg $mL^{-1}$ by Bio-Rad protein assay, it was reduced to 0.72 mg $mL^{-1}$ after purification with DEAE-Sephadex A-50 ion exchange chromatography (single peak, O.D = 0.715) and to 0.36 mg $mL^{-1}$ following purification with 2'5' ADP Sepharose 4B column chromatography (two peaks, O.D = 0.982 and 1.492).

The eluted protein fractions resulted from the different purifications methods was analyzed by 12.5% SDS-PAGE under reducing condition and showed only one band at 65 kDa which represents TGR as shown in lane 4 (Fig. 1).

**Reactivity of target antigen by indirect ELISA:** The antigenicity of the purified target antigen was tested by indirect ELISA technique. Samples of *S. mansoni* infected-human sera showed a strong reaction against SmTGR*?* antigen with mean OD = 2.092±0.201 and no cross reactions were recorded with sera of patients infected with other parasites e.g., *F. gigantica*, hydatid and hookworm (Table 1).

**Production of polyclonal antibody against TGR**

**Evaluation of anti-TGR polyclonal antibody level:** An increasing antibody level started 1 week after the first

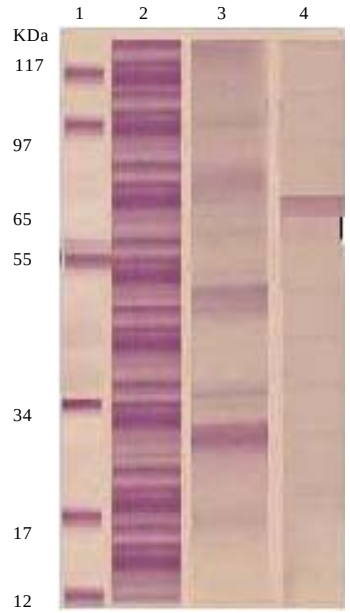


Fig. 1: Profile of eluted antigen of DOC extracts on 2'5' ADP Sepharose 4B column chromatography. Lane 1: Low MW standard. Lane 2: DOC extracts. Lane 3: Target antigen eluted from Sephadex A-50 exchange chromatography. Lane 4: Target antigen eluted from 2'5' ADP Sepharose 4B column chromatography

Table 1: Reactivity of purified target antigen by indirect ELISA

| Infection type | OD at 492 nm±SD |
|---|---|
| *S. mansoni* | 2.092±0.201 |
| *Fasciola* | 0.209±0.342 |
| Hydatid | 0.106±0.094 |
| Hookworm | 0.182±0.082 |

OD: Optical density; SD: Standard deviation

booster dose. The O.D recorded was 0.35 before immunization, 0.82 after priming dose and 1.86 1st boosters. Three days after the 2nd booster dose, immune sera gave a high titer against SmTGR antigen with OD of 2.97 at 1/250 dilution.

**Reactivity and specificity of anti-TGR polyclonal antibody:** Reactivity of anti-SmTGR pAb against SmTGR antigen and other parasite antigens (*F. gigantica*, hookworm, hydatid) was determined by indirect ELISA. The produced anti-SmTGR pAb gave a strong reactivity to SmTGR antigen. The O.D reading at 492 nm for *S. mansoni* was 2.84 compared to 0.262, 0.310, and 0.206 for *Fasciola*, hookworm, hydatid, respectively.

**Purification of rabbit anti-SmTGR polyclonal antibodies:** The IgG fraction of rabbit anti-*S. mansoni*

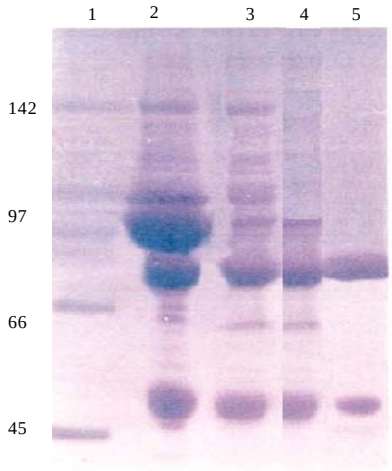


Fig. 2: Characterization of anti-*S. mansoni* polyclonal antibodies by 12.5% SDS-PAGE. Lane 1: Mw of standard protein. Lane 2: Crude anti-*S. mansoni* IgG pAb. Lane 3: Precipitated proteins after 50% ammonium sulfate treatment. Lane 4: Purified IgG pAb after 7% caprylic acid treatment. Lane 5: Purified IgG pAb eluted from Sphadex A-50 exchange chromatography

Table 2: Total protein content after different purification steps

| Purification methods | Protein content (mg $mL^{-1}$) |
|---|---|
| 1- Crude rabbit serum containing anti-*S. mansoni* pAb | 7.1 |
| 2- 50% ammonium sulfate precipitation | 4.2 |
| 3- 7% caprylic acid precipitation | 3.1 |
| 4- Ion exchange chromatography | 2.5 |

pAb was purified using different purification steps summarized with its protein contents in Table 2.

The eluted anti-SmTGR IgG obtained by DEAE Sphadex A-50 ion exchange column chromatography is represented by a single peak with maximum OD value 2.88 at fraction number 10.

**Characterization of anti-*S. mansoni* polyclonal antibodies by SDS-PAGE:** The precipitated proteins appeared as several bands. The purified IgG pAb was represented by H- and L-chain band at 53 and 31 kDa, respectively. The pAb appears free from other proteins (Fig. 2).

**Parasitological examination of human patients:** Parasitological examination and the intensity of *S. mansoni* infection in relation to ova count, according to stool analysis by MIFC method and Kato Katz quantitative. According to the intensity of infection, patients were classified into three subgroups:

- Light infection, included 40 patients with 10-50 ova (mean = 22±9.80)
- Moderate infection, included 20 patients with 50-90 ova (mean = 64±17.91)
- Heavy infection, included 70 patients with more than 90 ova with (mean = 98± 7.31)

In addition, 55 patients positive for other parasites classified to 10 fascioliasis, 15 echinococcosis, 15 ancylostomiasis and 15 ascariasis patients. Finally, 50 normal healthy individuals were included as control (Table 3).

**Detection of circulating SmTGR antigen in human sera:** Detection of SmTGR antigen in human sera infected with schistosomiasis and other parasites infected groups by sandwich ELISA in comparison to healthy control group, the cut off values for positivity was 0.312. Twelve cases out of 130 *S. mansoni* infected patient showed false negative results and the sensitivity of the assay was 90.77%. All healthy control samples were below the cut off value while 7 out of 55 patient infected of other parasites were above the cut off value giving an 87.27% specificity (Table 4).

**Detection of circulating SmTGR antigen in human urine:** Detection of SmTGR antigen in human urine infected with schistosomiasis and other parasites infected groups by sandwich ELISA in comparison to healthy control group, (the cut off values for positivity was 0.347). Seventeen cases out of 130 *S. mansoni* infected patient showed false negative results and the sensitivity of the assay was 86.92%. All healthy control samples were below the cut off value while 7 out of 55 patient infected of other parasites were above the cut off value giving an 87.27% specificity (Table 5).

Sensitivity, specificity, PPV, NPV and efficiency of sandwich ELISA used for detection of *S. mansoni* antigens in serum and urine samples of *S. mansoni* infected patients.

All data were collected, calculated according to specific equations and summarized in the Table 6.

Correlation between number of ova/gm stool and antigen level in serum and urine in human-infected with *S. mansoni*.

There was a strong positive significant correlation between number of ova/gm stool and O.D reading of ELISA for smTGR antigen level of *S. mansoni* infected human in serum samples (correlation coefficient r = 0.687; $p<0.001$) and in urine samples (r = 0.730; $p<0.01$).

## DISCUSSION

The study relies on the TGR unique enzyme as antigen detection in patients' serum and urine to evaluate its efficacy in the diagnosis of *S. mansoni* infection patients.

Table 3: Parasitological examination and the intensity of *S. mansoni* infection in relation to ova count

| | Parasitological examination | | | | |
|---|---|---|---|---|---|
| | Normal healthy | Schistosomiasis | | Other parasites | |
| | 50 | 130 | | 55 | |
| Class | | | | | |
| Number of individuals | - | Degree of infection | No. | Ova count (X ± SE) | Infection | No. |
| Subgroups | | Light 10-50 ova | 40 | 22±9.80 | Fascioliasis | 10 |
| | | Moderate 50-90 ova | 20 | 64±17.91 | Echinococcosis | 15 |
| | | Heavy over 90 ova | 70 | 98±7.31 | Ancylostomiasis | 15 |
| | | | - | | Ascariasis | 15 |

Data are represented as mean±standard deviation (SD)

Table 4: Detection of circulating SmTGR antigen in human sera

| | Positive cases | | | Negative cases | | |
|---|---|---|---|---|---|---|
| Groups | X (OD)± SE | No. | Positivity (%) | X (OD)±SE | No. | Negativity (%) |
| Healthy control (n = 50) | - | - | - | 0.29±0.01 | 50 | 100 |
| Schistosomiasis (n = 130) | | | | | | |
| Light infection (n = 40) | 0.76±0.24 | 35 | 87.5 | 0.25±0.11 | 5 | 12.5 |
| Moderate infection (n =20) | 0.98±0.31 | 18 | 90.0 | 0.20±0.23 | 2 | 10.0 |
| Heavy infection (n = 70) | 1.44±0.23 | 65 | 92.9 | 0.27±0.18 | 5 | 7.1 |
| Other parasites (n = 55) | | | | | | |
| Fascioliasis (n = 10) | 0.74±0.22 | 2 | 20.0 | 0.22±0.19 | 8 | 80.0 |
| Echinococcosis (n = 15) | 0.71±0.22 | 2 | 13.3 | 0.15±0.21 | 13 | 86.7 |
| Ancylostomiasis (n = 15) | 0.82±0.11 | 2 | 13.3 | 0.24±0.22 | 13 | 86.7 |
| Ascariasis (n = 15) | 0.65±0.15 | 1 | 6.7 | 0.21±0.11 | 14 | 93.3 |

X: Mean, OD: Optical density, SE: Standard error

Table 5: Detection of circulating *S. mansoni* antigen in human urine

| | Positive cases | | | Negative cases | | |
|---|---|---|---|---|---|---|
| Groups | X(OD)± SE | No. | Positivity (%) | X (OD)± SE | No. | Negativity (%) |
| Healthy control (n = 50) | - | 50 | - | 0.28±0.13 | 50 | |
| 100%Schistosomiasis (n = 130) | | | | | | |
| Light infection (n = 40) | 0.60±0.23 | 33 | 82.5 | 0.19±0.19 | 7 | 17.5 |
| Moderate infection (n = 20) | 0.82±0.22 | 17 | 85.0 | 0.24±0.12 | 3 | 15.0 |
| Heavy infection (n = 70) | 1.21±0.26 | 63 | 90.0 | 0.27±0.12 | 7 | 10.0 |
| Other parasites (n = 55) | | | | | | |
| Fascioliasis (n = 10) | 0.63±0.40 | 1 | 10.0 | 0.19±0.15 | 9 | 90.0 |
| Echinococcosis (n = 15) | 0.52±0.15 | 2 | 13.3 | 0.21±0.23 | 13 | 86.7 |
| Ancylostomiasis (n = 15) | 0.51±0.15 | 3 | 20.0 | 0.21±0.28 | 12 | 80.0 |
| Ascariasis (n = 15) | 0.50±0.17 | 1 | 6.7 | 0.30±0.19 | 14 | 93.3 |

Table 6: Key features in reliability of test results

| Specimen | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | Efficiency (%) |
|---|---|---|---|---|---|
| Serum | 90.77 | 87.27 | 94.40 | 80.00 | 89.73 |
| Urine | 86.92 | 87.27 | 94.16 | 73.84 | 87.02 |

Researchers focused on the TGR as a drug target where they demonstrate that TGR is an essential protein for the survival of *S. mansoni* in an aerobic environment using it as an effective redox mechanism for surviving reactive oxygen species from its host. In the same time, TGR involves a fusion of two proteins, Grx and TrxR, for performing all the redox activities. This dependence of *S. mansoni* on a single protein, TGR, for its protection from oxidative stress, makes it a promising drug target (Angelucci *et al*., 2010; Otero *et al*., 2010).

The researchers made large quantities of pure TGR and tested its activity against various substrates. It was found that, the enzymatic properties and substrate preferences of TGR differed somewhat from those of its mammalian counterparts (Kuntz *et al*., 2007). The previous features of the TGR should make it an attractive target for diagnostic antigen tool of schistosomiasis.

Studies revealed that, there are important differences between mammalian and parasite TGRs. First, the mammalian enzyme has a tissue-specific expression pattern and second, the Grx domain of mammalian TGR contains a CxxS (cysteine separated from serine by two other residues) active site motif, whereas the parasite TGRs contain a typical CxxC which is an active site motif that is found in most Grx (Agorio *et al*., 2003; Rendon *et al*., 2004). These differences suggest that, despite similar domain organization, mammalian and parasite TGRs may represent two functionally distinct types of proteins (Su and Gladyshev, 2004).

In this study the TGR purified from the *S. mansoni* was according to the method used by Maggioli *et al*. (2004). Affinity chromatography has been shown to be a very effective tool for isolation of candidate diagnostic and vaccine molecule (Fagbemi *et al*., 1995, 1997; Kumar *et al*., 2008) and so in the present study the principle of the affinity chromatographic method has been utilized for isolation of TGR antigen.

The DOC extracts obtained from adult *S. mansoni* worms contains 8 mg $mL^{-1}$ of total protein as measured by Bio-Rad protein assay, while it was 0.72 mg $mL^{-1}$ after purification with DEAE-sephadex A-50 ion exchange chromatography and 0.36 mg $mL^{-1}$ following purification with 2'5' ADP Sepharose 4B column chromatography. The yield of pAb was reasonable in comparison with the yield of purified Ig from any biological fluid following similar purification procedures (Yang and Harrison, 1996; El-Amir *et al*., 2008).

The eluted protein fractions resulted from the different purification methods were analyzed by 12.5% SDS-PAGE under reducing condition and showed only one band at 65 kDa, which represents TGR and this, is in agreement with Sun *et al*. (2005).

Critical steps in pAb production recommendation, injection and immunization protocol were according to Leenaars and Hendriksen (2005). An increasing antibody level started 1 wk after the first booster dose. Three days after the second booster dose, immune sera gave a high titer against SmTGR antigen with OD of 2.97 at 1/250 dilution. Among other factors, the dose and nature of the antigen as well as the route or kind of adjuvant used can ultimately dictate the outcome of an immunization. It may be possible that the simple alteration of antigen introduction and formulation can prevent an autoimmune response (Carvalho-Queiroz *et al*., 2004).

Reactivity of anti-SmTGR antibodies against SmTGR antigen and other parasite antigens (fasciola, hookworm, hydatid and trichostrongyloides) was determined by indirect ELISA. The produced anti-SmTGR pAb gave a strong reactivity to SmTGR antigen. The OD reading at 492 nm for *S. mansoni* was 2.84 compared to 0.26, 0.31, 0.21 and 0.28 for *Fasciola*, hookworm, hydatid and trichostrongyloides, respectively.

In the present study, the IgG fraction of rabbit anti- *S. mansoni* pAb was purified using different

purification steps including ammonium sulfate precipitation method followed by 7% caprylic acid precipitation method and finally ion exchange chromatography method (DEAE sephadex A 50 ion exchange column chromatography).

The total protein content of crude rabbit serum containing anti-*S. mansoni* pAb was 7.1 mg $mL^{-1}$. The yield of purified anti-*S. mansoni* IgG pAb following each purification step was determined by the assessment of protein content. Using the 50% ammonium sulfate precipitation method, the protein content was 4.2 mg $mL^{-1}$, while following 7% caprylic acid precipitation method the content dropped to 3.1 mg $mL^{-1}$. Finally, the protein content of highly purified anti-*S. mansoni* IgG pAb subjected to ion exchange chromatography method (DEAE Sephadex A-50 ion exchange column chromatography) was 2.5 mg $mL^{-1}$.

Collier *et al.* (2000) reported that, IgG is the most common immunoglobulin in the secondary immune response. In addition, Cook and Zumla (2003) reported that IgG is the predominant immunoglobulin after about 8-12 wk of infection.

The purity of IgG after each step of purification was assayed by 12.5% SDS-PAGE under reducing condition. Analysis of 50% ammonium sulfate-precipitated proteins by 12.5% SDS-PAGE, under reducing condition, showed that precipitated proteins appeared as several bands. The purified pAb IgG was represented by H- and L-chain band at 53 and 31 kDa, respectively. The pAb appears free from other proteins.

In the present study, the MIFC and Kato Katz methods were used as a background results to compare The results as researchers relied on these tests in their studies (Mengistu and Erko, 2007; Shane *et al.*, 2011; Xu *et al.*, 2011). The ultrasonography as a confirming was added, where the ideal confirmatory method should be independent of the first test method to increase the statistical reliability of the results (Xu *et al.*, 2011) The useful of ultrasonography as a confirming demonstrated by Rabello *et al.* (1994) where they stated that, two considerable advances were made towards the characterization of acute schistosomiasis, abdominal ultrasonography showed that liver and spleen enlargement and periportal and peripancreatic lymphadenomegaly are characteristic findings of acute schistosomiasis.

As schistosomiasis is often observed in conjunction with other infectious diseases (Rietveld *et al.*, 1987; Ghaffar *et al.*, 1991) and autoimmune antibodies (Abbas and Abdel, 1993) and because cross-reactive antibodies are frequently observed with other serological tests for schistosomiasis (Aronstein *et al.*, 1986; Correa-Oliveira *et al.*, 1988) a relatively large series of controls and other parasite patients were included in the present study. Assuming those test results as reference test, 130 positive samples with *S. mansoni,* 10 fascioliasis, 15 echinococcosis, 15 ancylostomiasis, 15 ascariasis patients and 50 normal healthy individuals were recorded for estimation of the potency of the pAb (in serum and urine samples) in the diagnosis of schistosomiasis.

Due to (according to the available knowledge), this study is the first validation of *S. mansoni* diagnosis by using TGR enzyme as antigen detection; we obligated to discuss this results with the result of other antigen in the same field.

The results of the present work revealed that, the ELISA test provided a sensitivity of 90.77% in detecting SmTGR antigen in serum samples with specificity of 87.27%. This data relatively agreed with sensitivity results revealed by Van Gool *et al.* (2002) which stated that, ELISA using *Schistosoma* egg antigen had a sensitivity of 92.0% in detecting egg-proven cases of schistosomiasis. In addition, it agreed with the results of several earlier studies in endemic and non-endemic areas (El Ridi *et al.*, 1986; Doenhoff *et al.*, 1993), but it is better than the sensitivity found in hospital study in the United Kingdom, 72%, recorded by Whitty *et al.* (2000).

Specificity is also one of the basic features of a diagnostic test that accurately identifies subjects without the targeted problem (disease) (i.e., specificity is expected to provide a minimum of false positives). Thus, a test with high specificity is preferable in areas with a high prevalence of disease (Mengistu and Erko, 2007). Specificity in ELISA study (87.27%) was less than that present in the study of Van Gool *et al.* (2002), who recorded 98.2% and this may be due to that the samples with other infectious diseases in this study live in endemic area where they may be undergo cryptic schistosomiasis, given the high prevalence of infection with *Schistosoma* in areas of endemicity. But in the previous study, it is sure to come from non endemic area, also both high and low specificity with ELISA have previously beenreported (El Ridi *et al.*, 1986; Tosswill and Ridley, 1986; Doenhoff *et al.*, 1993).

In comparison to the data revealed by the mAb of *Schistosoma* circulating antigen in serum and urine by sandwich ELISA, the sensitivity is slightly better than the present results of pAb and this was previously found by Nagy *et al.* (2010), where they had a sensitivity of 92.9% for serum and 90.5% for urine. But it is more best result in specificity (96% for serum and 94% for urine) higher than specificity result in this study (87.27% for both serum and urine) and this may be attributed to the relatively small number and variety of patients harboring parasites 30 individuals other than the parasite of infected group which is 42 *S. mansoni* infected patients. From the

previous study it can be suggested that, sandwich ELISA depends on pAb more accurate than fast dot-ELISA although it depends on mAb where its results in sensitivity and specificity recorded, 71.4 and 76% for serum and 76.2 and 64% for urine, respectively.

The specificities of the evaluated tests were mainly influenced by the cross-reactivity with specimens from fascioliasis, echinococcosis, ascariasis or ancylostomiasis patients. These observations suggested that when a positive result is obtained for a patient from an area co-endemic with schistosomiasis and one of the previous diseases, the history of infection with, or exposure to, the other two diseases should be considered also.

In making a choice between producing pAbs or mAbs, the desired application of the antibody and the time and money available for production should be considered. The fact that a pAb antiserum can be obtained within a short time (4-8 week) with little financial investment favors its use, whereas it takes about 3 to 6 month to produce mAbs. Many research questions can be answered by using a pAb antiserum. MAbs are specific for an epitope, which can be essential in specific cases (Lipman *et al*., 2005).

A study by Van Lieshout *et al*. (1995) suggested that, detection of CCA in urine using ELISA is as sensitive as the parasitological method in demonstrating low intensity of infection. Regardless obstruct of Stothard *et al*. (2006) and Mengistu and Erko, 2007), where they observed low sensitivity of urine CCA in detecting light infection with *S. mansoni* using the dipstick method. The present results recorded high sensitivity in the light infections in urine and in serum (82.5 and 87.5%, respectively) for detection of TGR antigen where in this high-prevalence, high-intensity area, the antigen tests were found to have a higher positivity score than the egg counts.

The prevalence of the disease could influence the predictive values. The higher the prevalence, the more likely a positive test would be predictive of the disease (Van Dam *et al*., 2004; Stothard *et al*., 2006). Thus, the positive predictive value of the detection of TGR antigen by ELISA, in both serum and urine, could be high in areas where the prevalence of *S. mansoni* is high as it had high results in the present study (94.40 and 94.16%, respectively), whereas it is low in low prevalence areas.

Xu *et al*. (2011) stated that, once transmission of the disease drops to very low levels, the PPV for any test is decreased. On the other hand, the NPV is expected to be low as the prevalence of the disease increases as observed in this study, where NPV recorded slightly low for serum and urine (80 and 73.84%, respectively).

The present study recorded PPV results higher than that recorded by Lin *et al*. (2008), which ranged from 20.8 to 24.6% while the NPV in the same study excelled the NPV in the present study and ranged from 93.1 to 94.4%. Therefore, the previous study revealed poor correlation between EPG (tested using Kato Katz method) and antibody level (detected by ELISA) in a high prevalence, low intensity *S. japonicum* infection village in china.

The present study revealed strong correlation between the TGR antigen detecting in serum and urine of *S. mansoni* infected samples and its intensity of infection and the number of the eggs excreted in stool detected by the backbone tests (MIFC and Kato Katz methods) where the OD reading of the ELISA test raised from light to the heavy infection in both serum and urine. Thus the present study exhibits strong positive correlation between number of EPG and OD reading of ELISA for TGR *S. mansoni* antigen level of *S. mansoni* infected human in serum samples (correlation coefficient $r = 0.687$; $p<0.001$) and in urine samples ($r = 0.730$; $p<0.01$). Similar findings had been reported by others (Van Lieshout *et al*., 1991; 1993; Nour El Din *et al*., 1994; Salah *et al*., 2000) in *S. mansoni* infected subjects. In addition, Hendawy *et al*. (2006) stated that, the level of Circulating Schistosomal Antigen (CSA) in serum and urine was positively correlated with the number of eggs excreted in stool of schistosomiasis patients, denoting the reliability of CSA detection as an indicator for intensity of infection and monitor of cure instead of using rectal snip invasive technique. Even though, it should be noted that the relationship between excreted eggs in stool and worm burdens is not always straightforward (Medley and Anderson, 1985; Stothard *et al*., 2011).

This study introduced a high diagnostic efficiency for the sandwich ELISA for detection of SmTGR antigen when applied to individuals with low or high worm burden in both serum and urine (89.73 and 87.02%, respectively). The diagnostic tool capable of detecting *Schistosoma* infections in its acute phase would be of great value permitting early treatment that could prevent the pathology associated with chronic infections (Bahgat *et al*., 2011). Thus, this study is in agreement with the believe of Shane *et al*. (2011), that points to contact testing of urine for schistosome antigen could be an effective tool in schistosomiasis mapping and control efforts. In addition, the results are in agreement with a recent study (Oniya *et al*., 2011) which shows that, there is a significant difference in the individuals with *S. mansoni* and those without, thus showing that this ELISA test actually does differentiate between individuals who have been infected and those who have not.

The importance of the choice of the circulating SmTGR antigen for detection by ELISA in this study, was revealed by a recent report (Oniya *et al*., 2011) which stated that, the sensitivity and specificity of ELISA in

schistosomiasis diagnosis has been documented, studies have shown different reports of the reliability of the egg-antigen ELISA based immunodiagnosis. In regard to Lin *et al.* (2008) study, which reported sensitivity ranged from 73.3 to 87.4%, with a lower specificity value ranged between 38.9 and 53.5% egg-antigen ELISA.

## CONCLUSION

In conclusion, this study revealed that, this antigen can play an important role in *S. mansoni* diagnosis. In the same time, further researches must be done on this unique enzyme in several diagnostic ways. At the first, the antigen must be tested to evaluate the specificity for distinguish between the different *Schistosoma* species and its sensitivity in non-endemic area. And the studies must be investigate ways to develop a rapid field-applicable test for the detection of this antigen in the urine and serum of schistosoma infected individuals, to overcome the clinical and direct parasitological problems and the restricted applies of ELISA in non furnisher laboratories. Where ELISA procedures remain relatively slow, even in an optimized and standardized format and they require skilled personnel and well-equipped laboratories.

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