World Journal of Medical Sciences 13 (1): 27-37, 2016
ISSN 1817-3055
© IDOSI Publications, 2016
DOI: 10.5829/idosi.wjms.2016.13.1.96189

Nano-Immunoassay for Diagnosis of Active Schistosomal Infection

[1]*Manal Kamel*, [1]*Hanan El-Baz*, [1]*Zeinab Demerdash*, [2]*Safaa El-Karaksy*,
[2]*Nancy El-Gendy*, [1]*Salwa Hassan*, [1]*Faten Salah*, [1]*EngyAbd El-Moneem*,
[3]*Mohamed Hendawy and* [3]*Ibrahim Aly*

[1]Department of Immunology, Theodor Bilharz Research Institute, Giza, Egypt
[2]Department of Clinical and Chemical Pathology, Faculty of Medicine, Cairo University, Cairo, Egypt
[3]Departments of Parasitology, Theodor Bilharz Research Institute, Giza, Egypt

Abstract: Sandwich ELISA using Gold nanoparticles (AuNPs) conjugated with anti-schistosomal monoclonal antibody (MAb) was evaluated for its sensitivity and specificity in diagnosing human *Schistosoma mansoni* infection. The study was conducted on serum samples of 116 subjects:71 *mansoni*-infected patients, (gp I), 25 patients infected with parasites other than schistosomiasis, (gpII) and 20 uninfected healthy individuals (gpIII). Patients in gp I were further subdivided according to egg count in their stool samples into light, modeate and severe infection. Results were compared with those after using MAb sandwich ELISA system. It was found that AuNPs-MAb/ ELISA reached a lower detection limit of 10 ng/ml compared to 85 ng/ml on using MAb/ ELISA and the optimal concentration of AuNPs-MAb used was 12 folds less than that of MAb. The sensitivity and specificity of ELISA using AuNPs –MAb for detecting circulating schistosomal antigen (CSA) were 100% & 97.8% compared to 87.3% &93.38 % respectively on using MAb/ELISA system. A more significant positive correlation was detected between ova count and CSA levels on using AuNPs–MAb/ELISA(r= 0.882) than with MAb/ELISA system(r= 0.770). In conclusion, loading AuNPs with MAb (6D/6F) increased the sensitivity and specificity of sandwich ELISA for detection of CSA, thus active and light infections could be easily detected. Moreover this binding will decrease the amount of MAb consumed in the assay and lower the cost. The significant positive correlation that was detected between ova count and OD reading in ELISA using AuNPs-MAb enables its use to detect the severity of infections and follow up of patients after treatment for monitoring of cure.

Key words: Nanoparticles · Monoclonal antibodies · Gold · Schistosomiasis · Circulating Schistosomal Antigen

INTRODUCTION

Schistosomiasis is one of the most prevalent human parasitic infections. It is a major source of morbidity affecting more than 207 million people in 76 countries. It was estimated that 97% of the infected cases are in the African continent [1, 2]. 120 million have symptoms, 20 million have severe illness [3] and a further 779 million people are at risk [4]. Diagnosis of schistosomiasis is, however, beset with several problems. The infections are generally chronic and characterized by the presence of parasite eggs in stool or urine. Hence routine diagnosis is microscopically, although it is of limited value because of inadequate sensitivity especially in light infections, <50 eggs/g feces for *S. mansoni* and <50 eggs/10 ml urine for *S. haematobium* [5, 6]. By time, antibody and antigen detection assays have been developed that led to an increase in sensitivity and specificity of these assays, especially the latter [7]. Consequently, many tests have been developed to detect different circulating schistosome antigens (CSA) in urine or serum samples of infected hosts, e.g sandwich ELISA [8-10] dot-ELISA [11] latex agglutination test [12] and dipstick assay [13-17] using either polyclonal or monoclonal antibodies (MAbs) [18-21]. So far, no immunodiagnostic assays have provided a "gold standard" for determining prevalence, identifying infected individuals for selective population chemotherapy, or assessing the effectiveness of

Corresponding Author: Manal Kamel, Departments of Immunology, Theodor Bilharz ResearchInstitute, Giza, Egypt.
E-mail: baher_ronny@hotmail.com.

intervention, including chemotherapy follow-up. However, the available antigen-based serological assays that detect schistosome-specific circulating molecules are also not sufficiently sensitive for early cases, especially those with a low intensity of infection [22, 23]. The use of nanoparticles promises to help promote in vitro diagnostics to the next level of performance [24, 25]. Loading of MAbs on gold nanoparticles combines the properties of the nanoparticles themselves (Larger surface area to volume ratio, which implies that they may render higher binding efficiencies) with the specific and selective recognition ability of the antibodies to antigens [26]. This study aimed at increasing the diagnostic efficiency of monoclonal antibody (MAb)-based antigen detection assays through gold nanoparticles conjugated with anti-schistosomal MAb (6D/6F), hence early and light *Schistosoma* infections could be easily detected. In addition, a decrease in the cost per test would be an important goal to be achieved.

MATERIALS AND METHODS

Preparation of MAbs

Revival of Cells and Propagation of Hybridoma Cell Lines: Three hybridoma cell lines of MAbs against tegumental *Schistosoma* antigens (AWTA) were prepared at the Immunology Department of TBRI and preserved in liquid nitrogen till further use [9]. Revival of cells was carried out under laminar flow according to Goding [26]. The frozen cells were thawed rapidly by immersing cryotubes in water bath at 37°C. Thawed cells were centrifuged at 200 xg for 5 min. at room temperature and were then resuspended in 1 ml serum-free medium (SFM).Cells were counted and diluted to a concentration of 20 x 10^4 cells/ml in growth media (SFM supplemented with 20% fetal calf serum (Hyclone), heat inactivated at 56°C for 30 min and seeded at 24 well culture plate (Becton Dickinson Labware, Bedford, MA). The cells were split every 48h and maintained in culture plates for about one week before transferring them to 25 cm^2 tissue culture flasks. Hybridomas were screened for anti-*S mansoni* tegumental antibodies by ELISA. Hybrids that were highly reactive were cloned by the limitation dilution, using splenocyte feeder layer according to Galfre and Milstein [27]. Hybridoma cells were injected intraperitoneally into BALB/c mice for ascites production.

Characterization of Monoclonal Antibodies

Assessment of Reactivity and Specificity of MAbs to Parasitic Antigens: The reactivity of MAbs against various parasitic antigens such as *S. mansoni* and *S. haematobium* SEA, extracts of *Fasciolagi gantica* and *Echinococcus granulosus* at concentrations of 30 µg (protein)/ml was determined by indirect ELISA as described by Mohamed *et al.* [19].

Isotypic Analysis of MAb: Determination of isotype of MAbs was done by indirect ELISA [28] using ELISA plates coated with 30 ug/ml of *S. mansoni* soluble egg antigen and a panel of anti-mouse immunoglobulin peroxidase conjugates (Goat anti- mouse IgM, IgG, IgG1, IgG2a, IgG2b, IgG3 and IgA) (Sigma Aldrich, Germany).

Identification of Target Antigens Recognized by MAbs: Using enzyme- linked immunoelectrotransfer blotting, Mab recognized 50 -65 kDa band of electrophoresed *S. mansoni* AWTA [9].

Purification and Conjugation of MAb: MAb was purified according to Garcia *et al.* [29]. The ascitic fluid containing IgM MAb was centrifuged at 1500 rpm 37°C for 15 minutes. The clear supernatant was collected in dialysis bags and soaked in 2% boric acid pH 6.0 at 4°C. The boric acid was changed every other day for 3 days. At the 4th day the supernatant was collected, ultracentrifuged at 100.000 xg at 4°C for 1 h. Supernatant was discarded and final precipitate was dissolved in a suitable amount of 0.2 M PBS, pH 7.2.MAbwas conjugated to horseradish peroxidase (Sigma Aldrich, Germany) following the periodate method according to Tijssen and Kurstak [30].

Gold Nanoparticles (AuNPs): Characterization and Functionalization: AuNPs were purchased from Nanotech Egypt Company for Photo-electronics-Dream Land with average diameter of 20 nm checked with a transmission electron microscope (JEOLI, JEM-2100). AuNPs were functionalized according to Omidfar *et al.* [31]. Briefly, 100ml tetrachloroauricacids (0.01% w/v $HAuCl4$) were brought to boiling and then a solution of 1% trisodium citrate was added with constant stirring. When the solution's color changed from light yellow to wine red, after about 8 min, the solution was cooled down. The pH was adjusted to 8.5 using 0.01 M potassium carbonate and 0.01% (w/v) sodium azide was added. The obtained solution was stored at 4°C in a dark-colored glass bottle for several months.

Loading of AuNPs to MAb: To prepare AuNPs-MAb according to Kumar *et al.* [32] 600ug of tegumental Mab; 30ug/ml, in phosphate-buffer (pH 7.5) was added to 20ml pH-adjusted AuNPs solution. The mixture was gently mixed for 3 h and subsequently 4ml of 10% BSA solution

was added to block the residual surface of the AuNPs. The mixture was then incubated for 20 min at room temperature before being centrifuged at 13, 000 rpm for 45 min at 4°C for three times. After the last centrifugation, the pellets were re-suspended in 2ml phosphate buffer (pH 7.2, 0.01 M) containing 1% BSA and 0.05% sodium azide. AuNPs-MAb was stored at 4°C before being used.

Optimization of Working Dilutions of Coating AuNPs-MAb and Determination of the Lower Detection Limit by Indirect ELISA [33, 34]: The prepared AuNPs-MAb was tested in different concentrations to obtain optimum dilutions by chequer-board titration against known positive and negative sera. A standard curve was set up using serial dilutions of *S. mansoni* SEA from 3ug/ml to 0.6ng/ml. Optical density (OD) readings at 492nm were plotted against the concentrations of the antigen preparations to determine the lower detection limit.

Application of Anti-*Schistosoma* MAb-Based Sandwich ELISA for Antigen Detection in Serum Samples of Schistosomiasis Patients

Study Population: This study was conducted on serum samples of 116 subjects. Seventy one patients (Male and female with age ranging from 20 to 50 years) infected with *S. mansoni* (Eggs were quantified in stool samples on 3 consecutive days by modified Kato' thick smear technique) [35] representing active infection with no other parasites including *S. haematobium* (gp I), 25 patients harboring parasites other than *Schistosoma* such as *Fasciola gigantica* and *Ecchinococcus granulosus* (gp II) and 20 age and sex matched healthy individuals were collected to serve as negative controls (gpÐI).Patients infected with *S. mansoni*(gp I), were further subdivided according to egg count in their stool samples into light {= 50 egg per gram(epg) (n= 17)}, moderate {51-100 epg (n= 33)} and severe infection {>100 epg(n= 21)}.

Ethical Agreement and Consent: This study was approved by the internal review board of Theodor Bilharz Research Institute, Cairo- Egypt for the use of the serum samples and data. Written informed consents were obtained from patients for the use of their data and publish the results of the study.

Detection of CSA in Serum Samples of Schistosomiasis Patients by Sandwich ELISA Using AuNPs-MAb: After several optimization trials, the following sandwich ELISA according to Engvall and Perlmann [28] and modified by Demerdash *et al*. [34] was performed for circulating antigen detection. Microtitration plates (Thomas Scientific) were coated with 100ul/well of AuNPs-MAb 1/12000 (250ng protein/well) in 0.1 M carbonate buffer pH 9.6 and left overnight at room temperature. Plates were blocked by the addition of 200µl/ well of 2.5 % fetal calf serum (FCS; Seromed) in 0.02 M phosphate buffered saline with 0.05% Tween 20 (PBS/T) pH 7.2 for 2h at 37°C.100ul of serum samples diluted 1/4 in diluent buffer were pipetted into the wells of the blocked plate in duplicate and incubated for 2h at 37°C. Plates were washed 3 times with PBS/T. 100µl of 1/1000 HRP-MAb in diluent buffer were added to each well and the plate was incubated for 1h at 37°C. The wells were then washed 5 times, 3 min each. The reaction was visualized by the addition of 100µl/well of O-phenylenediamine (Sigma) for 20 minutes in the dark at room temperature. The reaction was stopped by adding 50µl/well of $8NH_2S0_4$ and plates were read at 492 nm using an ELISA reader (Microplate Reader, Bio-Rad, Richmond, CA, USA).

Detection of Circulating Schistosoma Antigen (CSA) in Serum Samples of Schistosomiasis Patients by Conventional Sandwich ELISA Using MAb and HRP-Mab: Sandwich ELISA was done as previously described according to Demerdash *et al*. [34] using MAb at a dilution 1/1000 (3ug protein/well) for coating and HRP- MAbat dilution 1/500 for detection.

Statistical Analysis: Results are expressed as mean ± standard deviation (X ± SD) or number (%). Comparison between the mean values of variables was performed using One Way Analysis of Variance (ANOVA) and if significant results were obtained, Least Significant Difference (LSD) test as a post-hoc test was used. Pearson correlation coefficient was used to determine significant correlations between the egg count/g stool and ELISA OD readings. Receiver Operating Curve (ROC) was used to determine the sensitivity, specificity and accuracy of sandwich ELISA using MAb or AuNPs-MAb. The data were considered significant if p value was ≤ 0.05 and highly significant if p< 0.01. Statistical analysis was performed with the aid of the SPSS computer program (Version 16 windows).

RESULTS

Reactivity of Monoclonal Antibodies to *Schistosoma* Antigen by Indirect ELISA: After screening of different prepared MAbs, one MAb (6D/6F) raised against adult worm tegumental *Schistosoma* antigen (Anti- AWTA MAb) was chosen for this study, due to its high reactivity to *Schistosoma* antigen as it gave highest optical density (1.843 ±0.23) value.

Table 1: Reactivity of anti-*S.mansoni* adult worm tegumental antigen (anti- Sm AWTA) monoclonal antibody to *S. mansoni* adult worm tegumental antigen (AWTA), *S.mansoni* soluble egg antigen (SEA), *S.haematobium* SEA, *Fasciola* and *Hydatid* antigens compared to hybridoma growth medium

	ELISA mean OD values at 492 nm	
Coating antigen	Hybridoma growth medium	6D/6F Mab
S. mansoni AWTA	0.056	2.216
S. haematobium SEA	0.130	1.940
S. mansoni SEA	0.095	2.203
Fasciola antigen	0.071	0.254
Hydatid antigen	0.049	0.203

Production of Ascitic Fluid Containing MAb: Ascitic fluid was induced in 60 BALB/c female mice (8 weeks old) for MAb production by intraperitonial injection of 2x10^6hybridoma cells. Ten to 20 ml of ascitic fluid were obtained from each mouse containing 3 mg/ml of total protein as measured by Bio-Rad protein assay kit and were tested for the presence of specific anti-*Schistosoma* Ag antibodies by indirect ELISA. Ascitic fluid was highly reactive to *S. mansoni* SEA.

Characterization of (6D/6F) MAb: Isotyping of anti- AWTA MAbs was performed using Boehringer sub-isotyping kit. The produced MAb gave positive results with anti- IgM and with κ-type light chain. Target antigen of the prepared MAb was found to be glycoprotein in nature following periodate treatment. MAb reacted with repetitive epitopes on SEA when tested by immunoelectrophoresis.

Determination of 6D/6F Monoclonal Antibody Specificity: Specificity of MAbs was determined by ELISA against various parasitic antigens including *F. gigantica* and *Echinococcus granulosus* 6D/6F MAb was found to be strongly reactive with *S. mansoni* AWTA, *S. mansoni* SEA and *S. haematobium* SEA and did not show cross-reactivity with extracts of *F. gigantica* or *Echinococcus granulosus* Table (1).

Optimization of Working Dilutions of AuNPs-MAb: After chequer-board titration against known positive and negative sera, the optimal dilution of AuNPs-MAb was found to be 1:12000 (250ng/well) compared to 1:1000 (3ug/well) using MAb.

Detection of CSA in Serum Samples of Different Studied Groups by Sandwich ELISA Using Either AuNPs-MAb or MAb

Subject Groups: This study was conducted on the following subjects:

Group I: Patients infected with *S. mansoni* (Eggs were quantified in stool samples on 3 consecutive days by modified Kato' thick smear technique. Siongok *et al*. [35] representing active infection with no other parasites including *S. haematobium* (71 cases).

Group II: Patients infected with parasites other than *Schistosoma* (25 cases).

Group III: Uninfected age and sex matched healthy controls (20 individuals).

Sandwich ELISA: CSA levels were detected in sera of different studied groups by sandwich ELISA at OD readings equal to 492 nm using AuNPs-MAb for antigen capture and detection. The results were compared to those obtained by using MAb. The results were expressed as the mean (X) OD of each group ± standard deviation (SD) of the mean of the studied group. In order to measure the incidence of positivity for circulating *Schistosoma* antigen in the studied groups, it was necessary at first to determine the cut off point for positivity or the line of demarcation between positive and negative results. The cut off value was calculated as the mean OD readings of negative controls + 2 SD of the mean. The OD readings equal to or less than the cut off value were considered negative, while those readings greater than the cut off value were considered positive. It was found that serum levels of CSA using either AuNPs-MAb or MAb were highly significant ($P<0.01$) in *Schistsoma* infected group (gp III) compared to their corresponding levels in the other two groups gp II and gpI as shown in Table 2. No significant difference was detected in CSA level between other parasites group II and control group I.

Comparison of Immunological and Parasitological Results of Subject Groups

Results of Subject Groups Using AuNPs-MAb in Sandwich ELISA: The cutoff point used to determine a positive or negative serum sample was equal to 0.23. This value corresponded to 10 ng/ml of *S.mansoni* SEA in the standard curve. Positive CSA levels were detected in serum samples of all 71 *S. mansoni* infected patients (With positive *S.mansoni* eggs in their stool samples). Thus the sensitivity of antigen assay by sandwich ELISA using AuNPs-MAb was 100%. On the other hand, level of CSA in serum samples of all 20 healthy negative controls plus 24 out of 25 of other parasites group was found to be below cut- off value, giving an overall specificity of 97.8 % (Table 3, Fig. 1).

Table 2: Meanserum levels of circulating *Schistosoma* antigen (CSA) in the studied groups using eitherAuNPs-MAb or Mab. Data are presented as mean ± SD

	Study population		
Mab used	*Schistosoma*gp I (n= 71) Mean CSA	Other parasites gp II (n= 25) Mean CSA	Control gp III (n= 20) Mean CSA
MAb (6D/6F) (Cut off point=0.29)	1.12± 0.57[ab]	0.18 ± 0.05	0.21 ± 0.04
AuNPs-MAb (Cut off point=0.23)	1.02± 0.49[ab]	0.17 ± 0.05	0.17 ± 0.03

a p< 0.01 relative to control group.
b p< 0.01 relative to other parasites group.
p< 0.01highly significant.

Table 3: Comparative evaluation of sandwich ELISA OD readings using AuNPs-MAb and parasitological examination for diagnosis of S. *mansoni* infection

	Parasitology examination	
AuNPs-MAb	Control + Other parasites (n= 45)	*Schistosoma* (n= 71)
Negative (n= 44)	44 (97.78%) TN (True negative)	0 (0%) FN (False negative)
Positive (n= 72)	1 (2.22%) FP (False positive)	71 (100%) TP (True positive)

Data are expressed as number (percent).
TN:True negative TP:True positive FN:False negative FP:False positive

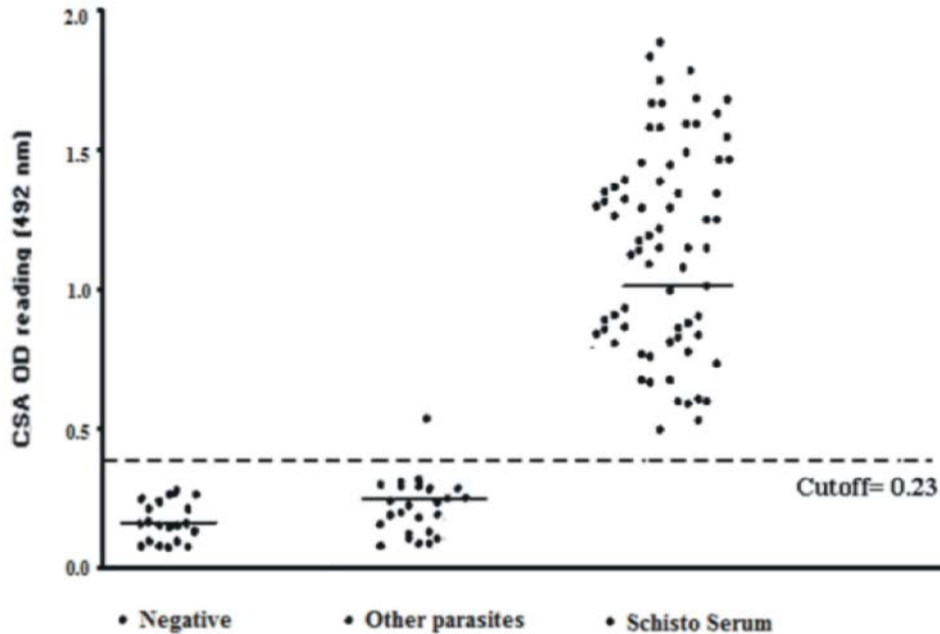

Fig. 1: Distribution curve showing (CSA) levels detected in sera of different studied groups measured as optical density (OD) values at 492nm using AuNPs-MAb in sandwich ELISA. Each small circle represents CSA level of a single patient. The horizontal line represents the cut-off value. CSA values below cut off were considered negative and values above were considered positive.

Results of Subject Groups Using MAb in Sandwich ELISA: The cutoff point used to determine a positive or negative serum sample on using MAb for detection of CSA levels of different studied groups was equal to 0.29. In this study, positive CSA levels were detected in sera of 62 out of 71 *Schistosoma* infected patients. Thus the sensitivity of antigen assay using MAb was determined 87.3%. On the other hand serum samples of all 20 healthy negative controls plus 22 out of 25 other parasites group (A total of 42 subjects out of 45) were negative for *Schistosoma* Ag and gave CSA levels below cut- off value, giving an overall specificity of 93.38% Table 4 and Fig. 2. It was observed that all nine schistosomal patients who were negative on using MAb /ELISA and positive with AuNPs-MAb/ELISA had an egg count below 50 epg feces (Light infection).

Table 4: Comparative evaluation of sandwich ELISA optical density (OD) readings using MAb and parasitological examination for diagnosis of *S.mansoni* infection

	Parasitology examination	
Mab	Control + Other parasites (n= 45)	*Schistosoma* (n= 71)
Negative (n= 51)	42 (93.33%) TN (True negative)	9 (12.68%) FN (False negative)
Positive (n= 65)	3 (6.67%) FP (False positive)	62 (87.32%) TP (True positive)

Data are expressed as number (Percent).

Table 5: Mean optical density (OD) readings of circulating *Schistosoma* antigen (CSA) levels in sandwich (ELISA) using either MAb or AuNPs-MAb classified according to intensity of infection in group (I)

		ELISA OD readings	
	Ova count (X ± SD)	Mab Cut off point=0.29	AuNPs-MAb Cut off point=0.23
Light infection ≤ 50epg (n= 17)	24.12 ±14.17	0.39 ± 0.19 (n=8) 0.25±0.027 (n=9)	0.54 ± 0.25 (n=17)
Moderate infection 51-100 epg (n= 33)	73.94 ±10.59	1.09 ± 0.29 (n=33)	0.95 ± 0.33 (n=33)[a]
Severe infection >100 epg(n= 21)	141.90 ±31.8	1.76 ± 0.31 (n=21)	1.53 ± 0.4 (n=21) [a]

Data are presented as mean ± SD.

[a] p< 0.01 relative to moderate and severe infection groups.

p< 0.01= highly significant

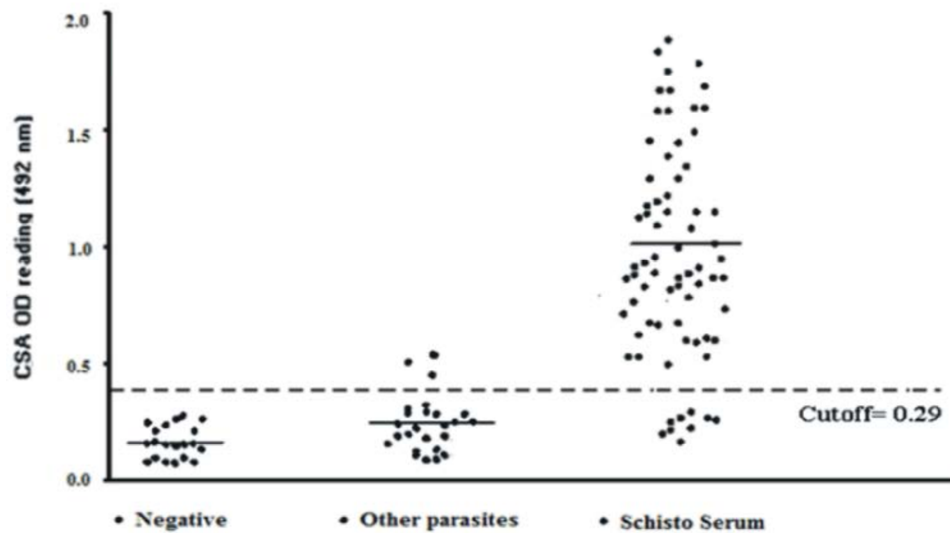

Fig. 2: Distribution curve showing (CSA) levels detected in sera of different studied groups measured as optical density (OD) values at 492 nm using MAb in sandwich ELISA. Each small circle represents CSA level of a single patient. horizontal line represents the cut-off value. CSA values below cut off were considered negative and values above were considered positive.

Relation between Intensity of Infection and CSA Levels in *S. mansoni* Patients: Patients infected with *S. mansoni* were classified according to egg count in their stool samples into light (Egg count ≤ 50 egg per gram (Epg)), moderate (Egg count 51-100epg) and severe infections (Egg count > 100 epg). Mean OD readings of CSA levels using either MAb or AuNPs-MAb in patients with light infection (0.39 ± 0.19 and 0.54 ± 0.25, respectively) was found to be significantly lower (p< 0.01) than their corresponding levels in both moderate (1.09 ± 0.29 and 0.95 ± 0.33) and severe (1.76 ± 0.31 and 1.53 ± 0.4) infections as shown in Table 5.

Correlation between Intensity of Infection and CSA Levels in *S. mansoni* Patients: A significant positive correlation was detected between ova count and OD readings using AuNPs-MAb (r= 0.882 and p=0.001) more than using MAb(r= 0.770 and p=0.001) in the *Schistosoma* infected group as shown in Fig. 3 & 4.

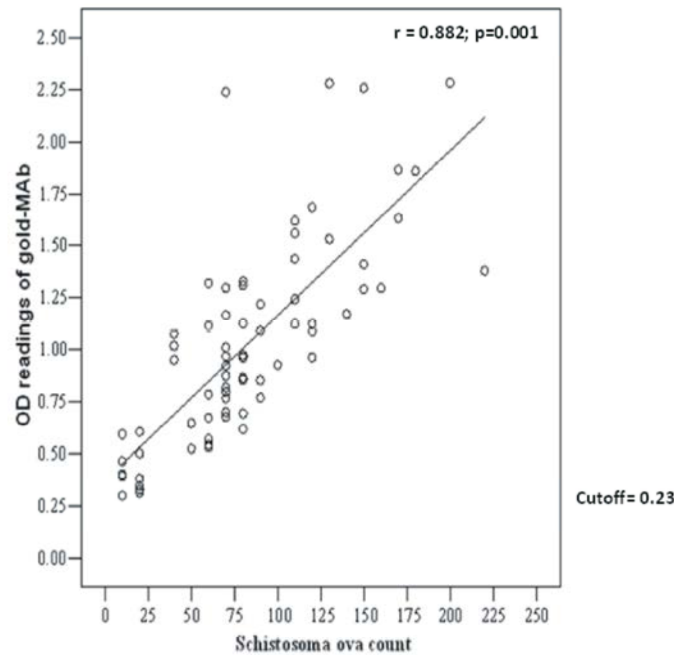

Fig. 3: Correlation between number of eggs per gram (epg) of feces and optical density (OD) readings of circulating Schistosoma antigen (CSA) using AuNPs-MAb in sandwich ELISA

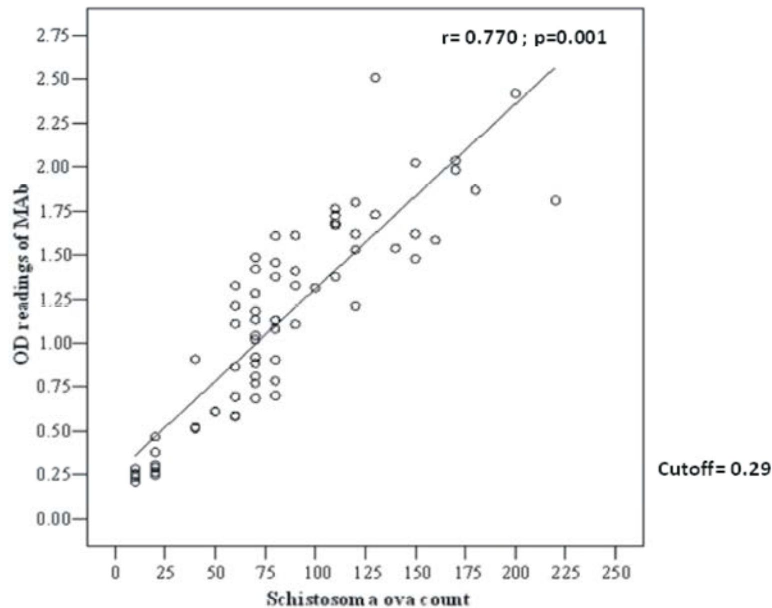

Fig. 4: Correlation between number of eggs per gram (Epg) of feces and optical denisty (OD) readings of circulating Schistosoma antigen (CSA) using MAb in sandwich ELISA

DISCUSSION

In Egypt, a national control program against schistosomiasis was started from 1993 to 2006 leading to a gradual decrease in the prevalence of the 2 schistosome species (1.9% for *S. mansoni* and 1.1% for *S. hematobium* [2]. Since then, schistosomiasis is considered as a neglected disease [1, 2]. By time, attention paid to schistosomiasis has been reduced leading to an increase of its prevalence again specially in rural areas reaching to 10% [36- 38]. The use of ELISA for detection of schistosomal soluble egg antigen is recommended by

many authors as a promising complementary field-applicable method for monitoring treatment and infection dynamics in endemic areas [17, 39]. This is an alternative to traditional parasitological approaches which became more fallible as both the infection prevalence and intensity diminished through control. CSA detection using anti- ATWA/ sandwich ELISA systems is now routinely used for diagnosis of active infection and monitoring of cure in our laboratory with a sensitivity 87.3% and specificity 93.38 %, for all in and out-patients at TBRI [9, 35, 40]. However, further efforts are still needed to increase the sensitivity of CSA assay especially for light and early infections.

This study aimed at increasing the diagnostic efficiency of monoclonal antibody (MAb)-based antigen detection assays through gold nanoparticles conjugated with MAb (6D/6F) which was chosen due to its high reactivity to S*chistosoma* antigens.

The prepared MAb was raised in ascites fluid, purified, HRP conjugated and applied in sandwich ELISA as both antigen capturing and HRP conjugated detecting MAb. Modified nanoparticles gold labeled MAb sandwich ELISA was performed as well, comparing its results with MAb-based sandwich ELISA. It was found that nanoparticles labeled -MAb/sandwich ELISA system reached a lower detection limit of 10 ng/ml compared to 85 ng/ml on using MAb – based sandwich ELISA and the optimal concentrations of nanolabeled MAb were found to be twelve folds less than the optimal concentrations of unlabeled MAb. Selection of gold nanoparticles in this study is due to the fact that they allow increased sensitivity by several orders of magnitude, extremely stable, have a long shelf-life. [41]. Modulation of the physicochemical properties of gold can be easily achieved by adequate synthetic strategies and give gold nanoparticles advantages over conventional detection methods currently used in clinical diagnostics [26].

In agreement with our results, Azzazy *et al.* [41] stated that conjugation of MAbs with gold decreases the amount of MAbs consumed and thus reduce cost for many diagnostic applications. Same result was found by Nagatani *et al*. [42] who showed theadvantage of decreasing the antibody concentration by using gold nanoparticles for detection of prostate-specific antigen in serum samples. These results are in accordance with that obtained by Tanaka *et al*. [43] who employed antibodies conjugated with AuNPs for detection of human chorionic gonadotropin hormone (HCG) antigen with a limit of detection reached 10pg/ml compared to 1ng/ml by conventional sandwich ELISA. In this study, the sensitivity and specificity of sandwich ELISA using AuNPs–MAb and MAb were 100% & 97.8% compared to 87.3% &93.38 %, respectively, on using MAb. The higher sensitivity and specificity detected in our work are in agreement with Ding *et al*. [44]. They stated that tests employing gold nanoparticles functionalized with antibodies enable high specificity and sensitivity by reducing background noise due to minimal non-specific binding of the gold nanoparticle, which in turn, creates an assay with 2-3 orders of magnitude more sensitive than conventional ELISA-based methods. Ahmed *et al*. [45] reported that using anti-*S. haematobium* antibodies coupled with nanoparticle for antigen detection by sandwich ELISA gave a sensitivity of 95% and 93.7% specificity compared to 87% and 87.3%, respectively, on using conventional microplate-based ELISA technique. It was observed that all nine patients with negative CSA levels byMAb and positive levels with MAb/AuNPs had an egg count below 50 epg feces (Patients with light infections). Conventional ELISA lacks sensitivity when testing light infections [9, 22] while gold nanoparticles conjugated to MAb are incredibly sensitive to detect nanomolar concentration of CSA. This finding supports the use of AuNPs- MAb as alternative to MAb in sandwich ELISA for detection of active and light infections.

CONCLUSION

Our data demonstrated that loading AuNPs with MAb (6D/6F) increased the sensitivity and specificity of sandwich ELISA for detection of CSA, thus active (Early) and light infections could be easily detected. Moreover this binding will decrease the amount of MAb consumed in the assay and lower the cost. The significant positive correlation that was detected between ova count and OD reading in sandwich ELISA using AuNPs- MAb enables its use to detect the severity of infections and follow up patients after treatment for monitoring of cure.

REFERENCES

1. WHO, 2012. Gearing up to eliminate schistosomiasis with increased donation of praziquantel from Merck KGaA. Geneva, Switzerland. World Health Organization.
2. King, C., 2006. Long-term outcomes of school-based treatment for control of urinary schistosomiasis: A review of experience in Coast Province, Kenya. Mem.Inst. Oswaldo Cruz., 1: 299-306.

3. Ross, A.G., P.B. Bartley and A.C. Sleigh, 2002. Schistosomiasis. N. Engl. J. Med., 346: 1212-1220.
4. Steinmann, P., J. Keiser and R. Bos, 2006. Schistosomiasis and water resources development: Systematic review, meta-analysis and estimates of people at risk. Lancet. Infect. Dis., 6: 411-425.
5. WHO, 2002. Prevention and control of schistosomiasis and soil-trans¬mittedhelminthiasis: Report of a WHO Expert Committee. Ge¬neva, Switzerland. World Health Organization. WHO Tech Rep Series, pp: 912.
6. Rudge, J., J. Stothard, M. Basáñez, A. Mgeni, I. Khamis, A. Khamis and D. Rollinson, 2008. Micro-epidemiology of urinary schistosomiasis in Zanzibar: Local risk factors associated with distribution of infections among schoolchildren and relevance for control. Acta. Trop., 105: 45-54.
7. Qian, Z.L. and A.M. Deelder, 1983. Schistosomajaponicum: Immunological response to circulating polysaccharide antigens in rabbits with a light infection. Exp. Parasitol, 55: 394-403.
8. Shaker, Z.A., M.A. Kaddah, S.B. Hanallah and M.I. El-Khodary, 1998. Production of monoclonal antibodies against target schistosomal antigen secreted in the urine of Schistosomamansoni-infected patients. Int. J. Parasitol., 28: 1893-1901.
9. Hanallah, S.B., S. Mohamed, K. Maher, N. Amer, H. Sayed, Z. Demerdash and Z. Shaker, 2003. A monoclonal antibody against tegument of adult *S. mansoni* worms detects active schistosomal infection. Kaser El Aini Med. J., 9: 209-221.
10. Salah, F., A. El Bassiouny, I. Rabia, Z. Demerdash, M. Roshdy and Z. Shaker, 2006. Human schistosomiasishaematobium: Effective diagnosis of active infection using a pair of monoclonal antibodies against soluble egg antigen. Parasitol. Res, 99: 528-533.
11. Ibrahim, R.B., S.H. Mohamed, Z.A. Demerdash, T. M. Diab, K. Maher, W. Safwat and Z.A. Shaker, 2010. Anti-S. mansonimAb-based latex agglutination: a reliable field applicable immunodiagnostic test for screening of active human schistosomiasis. J. Am. Sci., 6: 19-27.
12. Gezahegan, T., 1989. Development of a dipstick method for mosquito blood-meal identification and comparison with ELISA for potential field use. Med. Sei., 42: 593-600.
13. Stott, D.I., 1989. Immunoblotting and dot blotting. J. Immunol. Methods., 119: 153-187.
14. Van Dam, G.J., J.H. Wichers, T.M. Ferreira, D. Ghati, A. Van Amerongen and A.M. Deelder, 2004. Diagnosis of schistosomiasis by reagent strip test for detection of circulating cathodic antigen. J. Clin. Microbiol., 42: 5458-5461.
15. Hendawy, M.A., S.H. Mohamed and R.A. Ibrahim, 2006. Nitrocellulose cassettes (NCC) as a field applicable technique for rapid and effective diagnosis of schistosomiasis and fascioliasis. New. Egypt. J. Med., 35: 86-92.
16. Stothard, J.R., N.B. Kabatereine, E.M. Tukahebwa, F. Kazibwe, D. Rollinson, W. Mathieson, J.P. Webster and A. Fenwick, 2006. Use of circulating cathodic antigen (CCA) dipsticks for detection of intestinal and urinary schistosomiasis. Acta. Trop., 97: 219-228.
17. Van Lieshout, L., N. De Jonge, M.M. Mansour, S. Bassily, F.W. Krijger and A.M. Deelder, 1993. Circulating cathodic antigen levels in serum and urine of schistosomiasis patients before and after chemotherapy with praziquantel. Trans. R. Soc. Trop. Med. Hyg., 87: 311-312.
18. Nibbeling, H.A., L. Van Lieshout and A.M. Deelder, 1998. Levels of circulating soluble egg antigen inurine of individuals infected with Schisto-somamansoni before and after treatment with praziquantel. Trans. R. Soc. Trop. Med. Hyg., 92: 675-677.
19. Mohamed, S., Z. Demerdash, Z. Shaker, M. Mansour and A. Saad el Din, 1995. N. AbadeerProduction and characterization of a monoclonal antibody reactive with a repetitive epitope of concanavalin a boundglycoprotien fraction of Scistosomamansoni soluble egg antigen. Egypt Med. Associ., 78: 37-49.
20. Cai, Y.C., J. Guo, S.H. Chen, L.G. Tian, P. Steinmann, M.X. Chen, H. Li, L. Ai. and J.X. Chen, 2012. Chicken egg yolk antibodies (IgY) for detecting circulating antigens of Schistosomajaponicum. Parasitol. Int., 61: 385-390.
21. Demerdash, Z., S. Mohamed, M. Hendawy, I. Rabia, M. Attia, Z. Shaker and T.M. Diab, 2013. Monoclonal antibody-based dipstick assay: A reliable field applicable technique for diagnosis of Schistosomamansoni infection using human serum and urine samples. Korean. J. Parasitol., 51: 93-98.
22. Mott, K., H. Dixon, C. Carter, E. Garcia, A. Ishii, H. Matsuda, G. Mitchell, M. Owhashi, H. Tanaka and V. Tsang, 1987. Collaborative study on antigens for immunodiagnosis of schistomiasisjaponicum infection. Bull World Health Organ, 65: 233-244.

23. Doenhoff, M.J., J.G. Wheeler, K. Tricker, J.V. Hamilton, R.F. Sturrock, A.E. Butterworth, J.H. Ouma, G.G. Mbugua, C. Kariuki and D. Koech, 2003. The detection of antibodies against Schistosomamansoni soluble egg antigens (SEA) and CEF6 in ELISA, before and after chemotherapy. Ann.Trop. Med. Parasitol., 97: 697-709.
24. Azzazy, H. and M. Mansour, 2009.In vitro diagnostic prospects of nanoparticles.Clin.Chim. Acta, 403: 1-8.
25. Alharbi, K. and Y. Al-Sheikh, 2014. Role and implications of nanodiagnostics in the changing trends of clinical diagnosis. Saudi J. Biol. Sci., 21: 109-117.
26. Goding, J.W., 1986. Monoclonal Antibodies: Principle and Practice. Academic Press, Sydney Australia, pp: 56.
27. Galfre, G. and C. Milstein, 1981.Properties of monoclonal antibodies Strategies and procedures. Meth. Enzymol., 73: 3-46.
28. Engvall, E. and P. Perlman, 1971. Enzyme-linked immunosorbent assay (EL1SA). Quantitative assay of immunoglobulin G. J. Immunochem., 8: 871-874.
29. Garcia-Gonzalez, M., S. Bettinger, S. Ou, P. Olivier, J. Kadouch and P. Pourletty, 1988. Purlfication of murine IgG3 and IgM monoclonal antibodies by euglobulinprecipition. J. Immunol. Meth., 111: 17-23.
30. Tijssen, P. and P. Kurstak, 1984. Highly efficient and simple methods for the preparation of peroxidase and active peroxidase-antibody conjugates for enzyme immunoassays. Anal. Biochem., 136: 451-457.
31. Omdifar, K., S. Kia, S. Kashanian, M. Paknejad, A. Besharatie, S. Kashanian and B. Larijani, 2010. Colloidal nanogold- based immunochromatographic strip test for the detection of digoxin toxicity. Appl. Biochem. Biotechnol., 160: 843-855.
32. Kumar, S., J. Aaron and K. Sakolov, 2008. Directional conjugation of antibodies to nanopaticles for synthesis of multiplexed optical contrast agents with both delivery and targeting moieties. Nature Protocols., 3: 314-320.
33. Deelder, N., O. Boerman, Y. Fillie, G. Hilberath, T. Rotmans, M. Gerritse and D. Schut, 1989. Sensitive determinations of circulating anodic antigen in Schistosomamansoni infected individuals by an enzyme-linked immunosorbent assay using monoclonal antibodies. Am. J. Trop. Med. Hyg., 40: 268-272.
34. Demerdash, Z., S. Mohamed, Z. Shaker, S. Hassan, G. El-Attar, A. Saad-El Din, N. Abadeer and M. Mansour, 1995. Detection of circulating schistosome antigens in serum and urine of schistosomiasis patients and assessment of cure by a monoclonal antibody. J. Egypt. Soc. Parasitol., 25: 471-484.
35. Siongok, T.K.A., A.A. Mahmoud and J.H Ouma, 1976. Morbidity in schistosomiasismansoni in relation to intensity of infection: Study of a community in Machakos, Kenya. Am. J. Trop. Med. Hyg., 25: 273-284
36. Utzinger, J., G. Raso, S. Brooker, D. De Savigny, M. Tanner, N. Ornbjerg, B.H. Singer and E.K. N'goran, 2009. Schistosomiasis and neglected tropical diseases: Towards integrated and sustainable control and a word of caution. Parasitol., 136: 1859-1874.
37. Allam, A.F., 2012. Diagnosis of schistosomiasis in low endemic areas. In. Tech. Open. Book., 1: 1-10.
38. Stothard, J.R., J.C. Sousa-Figueiredo, C. Standley, G.J. Van Dam, S. Knopp, J. Utzinger, H. Ameri, A.N. Khamis, I.S. Khamis, A.M. Deelder, K.A. Mohammed and D. Rollinson, 2009. An evaluation of urine-CCA strip test and fingerprick blood SEA-ELISA for detection of urinary schistosomiasis in schoolchildren in Zanzibar. Acta.Trop., 111: 64-70.
39. Shaker, Z., H. Hassanein, R. Hamed, S. Botros, F. Mahmoud, S. Mohamed, A. El Garem, N. De Jonge and A. Deelder, 1992. Detection of circulating anodic antigen before and after specific chemotherapy in experimental murine schistosomiasismansoni. Int. J. Immunopharmacol, 14: 151-158.
40. Mena, M., P. Yanez-Sedeno and J. Pingarron, 2005. A comparison of different strategies for the construction of amperometric enzyme biosensors using gold-nanoparticles modified electrodes. Anal. Biochem., 336: 20-27.
41. Azzazy, H., M. Mansour and S. Kazmierczak, 2006. Nanodiagnostics: A new frontier for clinical laboratory medicine. Clin. Chem., 52: 1238-1246.
42. Nagatani, N., T. Yuhi, M. Chikae, K. Kerman, T. Endo, Y. Kobori, M. Takata, H. Konaka, M. Namiki and H. Ushijima, 2006. A sensitive immunochromatographic assay using gold nanoparticles for the semiquantitative detection of prostate-specific antigen in serum. Nano. Biotechnol., 2: 79-86.

43. Tanaka, R., T. Yuhi and N. Nagatani, 2006. A novel enhancement assay for immunochromatographic test strips using gold nanoparticles. Anal. Bioanal. Chem., 385: 1414-1420.
44. Ding, N., H. Zhao, W. Peng, Y. He, Y. Zhou, L. Yuan and Y. Zhang, 2012. A simple colorimetric sensor based on anti-aggregation of gold nanoparticles for Hg2+ detection. Coll. Surf: A. Physicochem.Eng. Aspects., 395: 161-167.
45. Ahmed, A., A. El Amir, I. Rabee and S. El Deeb, 2012. Effective diagnosis of schistosomiasishaematobium by immunomagnetic bead ELISA technique using super-paramagnetic nanoparticles. J. Am. Sci., 8: 833-841.