Effective diagnosis of *schistosomiasis haematobium* by Silver beads Sandwich ELISA

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**ABSTRACT**

Schistosomiasis is a major public health problem. Diagnosis by simple and rapid immunoassays is a priority. The magnetic bead immunoassay using magnetic nanoparticles conjugated with anti-schistosomal antibody was evaluated for diagnosing human schistosomiasis infection. The present study was to evaluate the sandwich ELISA as a simple test for the detection of schistosomal antigen (CSA) in serum and urine samples of *S. haematobium* patients and compare it with ELISA. Investigation conducted on eighty six cases divided to three groups, 34 were positively for *Schistosoma haematobium*, 32 were positively for intestinal parasites ova and negative for *S. haematobium* ova in urine and 20 were negative urine and stool examination (Control). Immunomagnetical bead based Enzyme-linked immunosorbent assay (ELISA) using for detected for antigen in sera and urine infected by *S. haematobium*. Sandwich ELISA sensitivities was 79.4% (serum) and 73.5% (urine) and which increase by used nano-sandwich ELISA to 88.2% (serum) and 82.4% (urine), respectively. Sandwich ELISA specificities was 86.4% (serum) and 80.8% (urine) and which increase by used nano-sandwich ELISA to 93.3% (serum) and 88.5% (urine). We found that, nano-sandwich ELISA assay had highly sensitive and specifically and technical method was applicably, fast, cheaper, accurate and promising diagnostic method for schistosomiasis.

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**INTRODUCTION**

Schistosomiasis considered as one of the fundamental word related ailments obtained by man through exercises related with freshwater, for example, cultivating, washing, washing and amusement. It has been additionally perceived as an ailment of critical financial and general wellbeing significance second just to jungle fever. Urinary schistosomiasis stays significant wellbeing trouble in ailment endemic zones of Africa and the Middle East particularly Egypt, influencing in excess of 110 million individuals in provincial, agrarian, and peri-urban zones ([Abdel-Wahab et al., 2000](#)). In Egypt, urinary schistosomiasis is nevertheless representing a serious fitness problem to deal with. Due to manage programs over the remaining decade, a decline in the occurrence of human schistosomiasis in Egypt has been reported, however the disease is still endemic in many foci ([Contis and David, 1996; Al-Sherbiny et al., 1999](#)). Detection of schis-
tosomal circulating antigens looks to be an effective method for discriminate among preceding exposure and current infections. Schistosome antigens detecting as alternative to egg counting in urine (El-Shafei et al., 2002; Wilson et al., 2007). *S. haematobium* infections, egg antigens might need to more establishing in full-size amounts even when eggs counts degrees have been very low, Live eggs exercise of trapped in bladder wall (Bottieau et al., 2006; Demerdash et al., 1995). Sandwich ELISA technique are among the most commonly used assays in detection of schistosomal antigens proved to more sensitively and specifically for antigen detection either in serum or in urine (Waugh et al., 2007). Detection of schistosomal circulating antigens appears to be an high quality method for discriminating among pre-exposure and contemporary infections. Schistosome antigens detection of taken distinguished area and may additionally be regarded as an alternative to egg counts in urine (Kahama et al., 1999). *S. haematobium* infected, eggs antigens ought to be validated in quantity even egg rely stage was very low, might be related to live eggs activities trapped in bladders wall (Doenhoff et al., 2004). Immuno-magnetic beads were uniform, polymer particles coated by polystyrene shell which provided smooth hydrophobic surface to facilitate physical absorption of molecules (Gessler et al., 2006; Gunderson et al., 1992; Conlan et al., 2009). Our target is evaluating prepared *S. haematobium* antigen roles in detecting active schistosomal infections throughout raising anti-*S. haematobium* pAb using sandwich and nano-ELISA.

**PATIENTS AND METHODS**

**Study population**

Our infestation conduct on *S. haematobium* infected cases from many endemic places in Egypt (El Fayoum, El-Sharkia and Kafr-EL-Shekh). The study was held in the period from April 2016 to June 2017. In the present study, a number of 86 individuals were enrolled. All cases and healthy volunteers subjected to medical and repeated parasitological stool examinations using Kato-Katz approach (Kongs et al., 2001), Merci-thiolate-Iodine-Formaldehyde concentration (MIFC) method and formal ether attention strategies (Knight et al., 1976). Urine evaluating perform for all groups and using sedimentation approach (Lier et al., 2009), nucloepore filtration techniques (Lengeler et al., 1993). Ethical problems handled in accordance to International Ethical Guidelines for Biomedical Research. Group A, *S. haematobium* infected (34), based on presences of Schistosoma egg in urine. Group B, Other parasites than Schistosoma (32). Group C medical staff at Theodor Bilharz Research Institute (TBRI) served as parasite free-healthy negative control (20).

**Preparation of antischistosomal worm antigen preparation polyclonal antibodies (Anti-SWAP pAbs)**

Two rabbits were injected intramuscularly (i.m.), with 100 μg of schistosomal worm antigen preparation (SWAP) with equal volumes from Freund’s adjuvant. Then, 3 booster doses 0.5 mg SWAP with equal vol. of incomplete Freund’s adjuvant give at 1 week. 1 week later; last booster dose, rabbit’s sera obtained and polyclonal antibody (pAb) fractions were purity using 50% ammonium sulfate precipitation (McKinney and Parkinson, 1987). More purifications from pAb by 4% caprylic acid.

**Preparation and characterization of purified SWAP.**

Adult clean *schistosoma* worms homogenized in 2 vol. from 20 mM Tris-Hcl buffer and centrifuged at 30,000 rpm / 30 min. Entire homogenization and centrifugation process was performed at 4°C. Supernatant fraction were decant and assay to determination protein and store later on –20°C till using. SWAP purify from the CPE by combination of ion-exchange chromatography on diethyl aminoethyl (DEAE)- Sephadex A-50 and gel filtration by Sephacryl high resolution (HR)-200. Absorbances of every fractions were assessment at 280 nm and purified of production protein by sodium dodecyl sulfate polyacrylamide gel electrophoresis u der reducing condition (Laemmli, 1970).

**Sandwich ELISA**

The reactivity of anti-SWAP was explored using Sandwich ELISA. S-ELISA test based on the original method (Engvall and Perlmann, 1971) was used with microplate modification (Elkawaz and Ghaffarifar, 2009). Plates coat by 100 μl/well from pAb anti-SWAP (20 μg/ml) in carbonate buffer (0.06M, pH 9.6) and incubating overnight. Plates wash thrice by 0.1 M phosphate buffered saline/ Tween-20 (PBS/ T), and wells block by 200 μl of 2.5% FCS and incubating on 37°C / 2 hr. Washing 3lates tribal times and 100μl (serum) adding to every well and incubating (2 h /37°C) then 100 μl of peroxidase-conjugated anti-SWAP pAb dilute 1/250 add and incubation (1 hr). 100 μl from freshly prepared substrate solution dispense in every well / 5 min., then 50 μl from stopping buffer add for stopping enzyme-substrate over reaction. Absorbance measure at 492 nm by ELISA reader. Cut off values calculated as average OD reading of negative control ± SD.
Magnetic Microbeads Based-Sandwich ELISA

Microtitration plates coat by using 100 μl/well of anti-
S. haematobium antibodies coupled with mag-
netic microbead nanoparticle (5 μg/ml) in carbon-
ate buffer 0.06 M, pH 9.6 overnight. Washing plates
treble times with 0.1 M PBS/T. Remain sites in well
was block by 200 μl/well from 2.5% FCS/PBS/T and
incubating/2 hr/37°C. Washing plates 3 times with
PBS/T. 100 μl of serum pipette to wells in dupli-
cate and incubating (2 hr /37°C) washing wells
treble. 100μl/well of peroxidase-conjugated poly-
clonal antibodies of1/3000 add and incubating 1
hr (Aly et al., 2013).

Statistics

Data analyses by use student's t-test. Standard formu-
las used to estimating sensitivities, specificities,
positive and negative predictive ratio, also, false
positive and negative rate, P< 0.05 (Snedecor and
Cochran, 1991).

RESULTS

Preparation of S. haematobium SWAP antigen

Purification of S. haematobium SWAP antigen was
shown in Figure 1. The eluted antigen following
purification by DEAE sephacryl S-200 gel filtration
column chromatography is single peak with max.
Optical Density (OD) equal to 1.25 at fraction num-
ber 10 (representing the fraction with the highest
protein content).

Figure 1: Elute profile for the purified SWAP
antigen by gel filtration chromatography

Characterization and reactivity of SWAP antigen

Eluted protein fraction result from different purifi-
cation methods analyzed by 12.5% Sodium Dodecyl
Sulfate-Polyacrylamide Gel Electrophoresis (SDS-
PAGE) under reducing conditions and given differ-
ent protein Major bands at 100, 95, 75, 67, 45 and
29 KDa with many minor bands (Figure 2).

Lane 1: Low molecular weight standard.

Lane 2: Crude schistosomal antigen.

Lane 3: Partially purified after homogenization.

Lane 4: Partially purified after ultrafiltration unit.

Lane 5: Target antigen eluted from sephacryl S-200.

Detection of S. haematobium SWAP antigen in
serum samples

The anti-S. haematobium SWAP IgG-pAb were using
to detecting S. haematobium SWAP in human sam-
ple serum and urine and employed as antigen cap-
ture and HRP pAb as conjugate in sandwich ELISA.
The sandwich ELISA technique was used for prelim-
inary standardization and optimization of different
materials concentrations and dilutions before the
application of the technique on humans’ era.

Cut off values were 0.302 through S. haematobium
SWAP delection antigen in serum, the results
showing that 27 of 34 were positive cases (79.4
%) of group infected with S. haematobium, while
7 of 34 were negative cases (false negative result)
(20.6%). In cases with other parasitic infection only
three 3 had positively by Fasciola, 2 by H. nana, 2
by Ascaris and no positive result 1 by Hookworms,
25 cases were negatively. Healthy control are nega-
tively (Table 1).

Detection of S. haematobium SWAP antigen in
serum samples by Nano-sandwich ELISA

The calculated cut off OD value was 0.225. The
presence of S. haematobium SWAP antigen in serum
samples was evaluated using nano-silver sandwich
ELISA method and OD value of S. haematobium
infected group (1.02±0.18) significant higher com-
paring to parasites group.

30 given positive results from 34 cases and only 4
gave false negative. Assay sensitivities was 88.2%.
20 healthy control negatively being below cut off val-
ues of SWAP positivity give 100% specificity of pro-
cedures, comparing to infected group. In group B, 4
cases were positively (2 with Fasciola, 1 with Ascaris
Table 1: Results of *S. haematobium* SWAP antigen detection in serum samples by sandwich ELISA

| Groups          | Positive cases | Negative cases |
|-----------------|----------------|----------------|
| Healthy control | 20             | 0.235±0.07     |
| *S. haematobium*| 27             | 1.12±0.19      |
| *Fasciola*      | 3              | 0.429±0.02     |
| *H. nana*       | 2              | 0.615±0.06     |
| *Ascaris*       | 2              | 0.701±0.05     |
| *Hookworms*     | 0              | 0.205±0.08     |

Table 2

The incidence of positivity

The sensitivity and specificity of the two techniques using to detected *S. haematobium* SWAP in human sera, where use nano sandwich ELISA increase the sensitivity and specificity of the techniques. The sensitivity of sandwich ELISA was 79.4% in serum and it increases by used nano sandwich ELISA to become 88.2% in serum. The specificity of sandwich ELISA was 86.6% in serum and it increases through use nano-sandwich ELISA to become 92.3% in serum (Table 3).

DISCUSSION

In order to evaluate the different immunodiagnostic antigen detection assays, selection of a proper antigen and its purification followed by propagation of its specific antibodies and purification were mandatory. Comparative evaluation of *S. haematobium* SWAP antigen detection with sandwich ELISA relation to nanodiagnostic assays sandwich enzyme-linked immunosorbent assay based on immunomagnetic beads (IMB-ELISA) techniques was performed. SWAP antigen was used in the present study because using soluble egg antigens (SEA) and crude worm antigen have many drawbacks. The crude worm antigen shows weak immunogenicity due to few T cell epitopes and also its inability to induce lymphocytic proliferation of humans in early stage schistosomiasis (*Parizade et al., 1994; Ondigo et al., 2010*). Also, SEA shows immunoregulation and suppression as the disease persists and progresses, so it may not be reliable also in late stages (*Ridi et al., 1997; Chand et al., 2010*). Standardization of sandwich ELISA used for detection of SWAP antigen was carried out before the application of the technique on human samples.

The purified IgG fraction of the rabbit sera was employed as both antigen capture and peroxidase conjugated detecting antibody. On detection of *S. haematobium* SWAP antigen in human serum samples by sandwich ELISA; 7 out of 34 *schistosomiasis haematobium* cases (group A) gave positive results with 79.4% sensitivity. In healthy control group (group C), no positive results were obtained giving a specificity of 100% compared to this group. While 7 cases out of the 30 patients with other parasitic infection (group B) showed positive results giving a specificity of 86.0%. Correlation was positively among *S. haematobium* eggs number in 10 ml urine and SWAP levels in sera of *S. haematobium* infect using sandwich ELISA.

Similarly, *Salah et al. (2006)* detected SEA in serum samples of *S. haematobium* infected patients. They reported that the procedure gave a sensitivity of 89% and a specificity of 100%. also, in agreement with the current study, *Elkawaz and Ghaffarifar (2009)* detected adult worm antigen in *S. haematobium* infected serum by use ELISA and showed a sensitivity of 82% and specificity of 95%. On the other hand, *Stothard et al. (2009)* used SEA-ELISA for detection of *S. haematobium* infections. The performance of the SEA-ELISA using sera showed a sensitivity of 89% and a specificity of only 70%.

In this study, concerning detection of SWAP in human samples with sandwich ELISA using nanomagnetic beads (sandwich IMB-ELISA). In serum samples, 30 out of 34 *schistosomiasis haematobium* cases (group A) gave positive results with 88.2% sensitivity. In healthy control group (group C), no
Table 2: Results of *S. haematobium* SWAP antigen detection in serum samples by nano-sandwich ELISA

| Groups              | Positive cases (n) | Positive OD (X± SD) | Negative cases (n) | Negative OD (X± SD) |
|---------------------|--------------------|---------------------|--------------------|---------------------|
| Healthy control     | 20                 | -                   | 20                 | 0.244±0.06          |
| *S. haematobium*    | 30                 | 1.29±0.16           | 4                  | 0.202±0.05          |
| *Fasciola*          | 2                  | 0.537±0.05          | 6                  | 0.211±0.03          |
| *H. nana*           | 0                  | -                   | 8                  | 0.181±0.03          |
| *Ascaris*           | 1                  | 0.719±0.15          | 7                  | 0.188±0.03          |
| *Hookworms*         | 1                  | 0.921±0.16          | 7                  | 0.219±0.08          |

Table 3: Sensitivity and specificity percentage of sandwich ELISA and nanosandwich –ELISA in serum

| Techniques                 | %Sensitivity Serum | %Specificity Serum |
|----------------------------|--------------------|--------------------|
| Sandwich ELISA             | 79.4%              | 86.6%              |
| Nano-Sandwich ELISA        | 88.2%              | 92.3%              |

positive results were obtained giving a specificity of 100% compared to this group. While only 4 cases out of the 30 patients with other parasitic infection (group B) showed positive results giving a specificity of 92.0%.

**CONCLUSION**

Conclusion use of nano bead immunoassay offered the potential advantage of sensitivity and specificity of the assay that may be due to the use of magnetic beads which can utilize larger surface area with highly binding capacities and rapidly reactions kinetics of solution with simples separation of bound and unbound materials on solid phases and provide good chance of enhance sensitivity of antigen detecting. The IMB-ELISA appears to be a sufficiently sensitive and feasible assay for detection of schistosomal antigenemia.

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**Conflict of Interest**

The authors declare that there is no conflict of interest for this study.

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