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Evaluation of circulating antigen in urine technique for diagnosis of *Schistosoma haematobium* infection

Ahmed Abdelhalim Yameny

Society of Pathological Biochemistry and Hematology, Egypt

Ahmed A. Yameny (Email: dr.ahmedyameny@yahoo.com)

ABSTRACT

Background : Diagnosis of urinary and intestinal schistosomiasis by detecting circulating *Schistosome* antigens in serum and urine have been developed and assessed to dissolve problems with parasitological diagnosis in low endemic areas or for early diagnosis, this study aimed to evaluate commercially available kit, using circulating antigen in urine as alternative for microscopic testing of urine to diagnose *S.haematobium* infection **Subjects and methods:** This study is a cross-sectional study designed to evaluate circulating antigen a commercially available kit was used for determine urinary schistosomiasis in the study population, this performed on 50 positive *S.haematobium* samples and 50 negative samples for individuals matching in age and sex , in addition, urine microscopic examination was done for detection of *S.haematobium* eggs by sedimentation centrifugation and nuclepore filteration techniques. **Results :** The results showed that circulating antigen kit in urine had a sensitivity of 56% and specificity of 76%.. PPVs was 70% whereas NPVs was 63.3 %. As for diagnostic efficiency, it was 66%, where the area under the curve (AUC) was sufficient 0.63 . **Conclusion and Recommendations:** Years ago this commercial kit used in this study and in all medical laboratories in Egypt for detection of schistosome antigen in urine is called schistofast bilharzial antigen in urine , this study showed low sensitivity (56%) and lowe specificity (76) than expected resuls for detection of circulating antigen and they are generally more expensive than microscopic examination.

Keywords: *Schistosoma haematobium***,**CAg,circulating antigen , validity, urinary schistosomiasis.

1.Introduction

Diagnosis of schistosomiasis is usually performed by parasitological(microscopic detection of eggs), and/or immunological methods(antibody and antigen detection)[(1)]**.** Methods have been developed for the diagnosis of light infections, which developed on either detection of antibodies specific to schistosome antigens or the presence of schistosome circulating antigens (SCA) in patients (Salah et al., 2006) [(2)] . Schistosome antigens are present in serum and urine of infected subjects [(3)] . According to their migratory behavior in immunoelectrophoresis they are commonly

referred to as circulating anodic antigens (CAA) and circulating cathodic antigens (CCA). These two circulating adult worm antigens are the basis of antigen capture immunoassays [(4)] . Somatic schistosome antigens, such as circulating anodic antigen and circulating cathodic antigen, can be detected and quantified with labelled monoclonal antibodies in serum or urine of infected individuals [(5)] , many attempts have been made to identify the egg antigens which are responsible for inducing those reactions and which proved also to be useful immunodiagnostic reagents (McManus and Loukas, 2008) [(6)], These tests can differentiate between past and active infections, as the circulating antigens are probably present only when there is active infection (Doenhoff 2004)[(7)], circulating antigens are released from living worms, antigen levels may correlate directly with parasite load, whilst microscopy does not. This may make the circulating antigen test useful in monitoring the dynamics of worm burdens and clearance of worms after treatment(Cavalcanti 2013; Rollinson 2013)[(8,9)].

during the acute phase or in recently reinfected cases of schistosomiasis, immature worms may produce worm antigens(e.g., CCA) before eggs are excreted , this process may result in a positive result using the
circulating antigen assay and a negative result using microscopic technique for eggs[(10,11)]. The level of antigen correlate well with the intensity of infection and are rapidly cleared from the circulation following successfull treatment [(12,13)] .Feldmeier and others measured CAA and CCA levels four months post-treatment and their results showed a significant decrease in serum CAA and CCA after treatment with praziquantel,but they related this decrease to infection with *S.mansoni* and not to *S.haematobium* [(14)] . The main advantage of antigen detection,and particularly CAA detection in serum ,is the fact that antigen levels show little fluctuation. A one-point determination therefore provides more reliable quantitative data than in the case of a parasitological diagnosis [(15)] .

On the otherhand, the main disadvantages of antigen detection are related to the availability and cost of the reagents,and to relatively time-consuming and expensive (ELISA) assay, which also is not suitable to use outside a laboratory setting [(15)] .

Questionnare and chemical reagent strip for haematuria and proteinuria can considered for the diagnosis *of S. haematobiu*m where microscopy is unavailable In areas with high prevalence of infection[(16,17)], recently Schistosomiasis infection was decreased in many countries and has been eliminated in Iran, Lebanon, Morocco and Tunisia with absence of new recorded cases in the past few years (WHO, 2007)[(18)]. In Egypt (2016), due to different control measures, the overall prevalence *S.haematobium* and *S.mansoni* fell down to less than 0.2% , and Egypt has started of a campaign to reach the final elimination of schistosomiasis by 2020 [(19)]. So many medical laboratories in Egypt using a commercially available kit by circulating antigen (Schistofast ABC Diagnostic), this study aimed to evaluate commercially available kit, using circulating antigen in urine as alternative for microscopic testing of urine to diagnose *S.haematobium* infection.

2. Material and methods

2.1. Study population and ethical consideration

This study included 100 patients attending Ministry of health laboratory centers, in El-Fayoum Governorate , this study subjects were randomly selected irrespective of the age-group and both sex were included. All the studied population were informed about the purpose of samples collection and their consents were obtained . Patients were free to refuse sample collection.

2.2. Study design

This research is a cross-sectional study designed to evaluate circulating antigen by a commercially available kit (Schistofast ABC Diagnostic, New Damietta city ARE) for determine urinary

schistosomiasis in the study population. This study was targeting customers who came for laboratories of health centers for urine analysis. This was performed on 50 positive *S.haematobium* samples and 50 negative samples for individuals matching in age and sex.

2.3. Collection and processing of urine samples

Clean specimen bottles were labeled with the needed information and issued to the participating individuals whose informed consent was sought earlier , each patient was given a wide mouth screw-capped container into which to void urine . This was carried out between 10.00 am and 2.00 pm when ova count of *S.haematobium* is expected to be at its peak[20]. In this study, urinary schistosomiasis was defined as the presence of ova of *S.haematobium* in the urine.

2.4. Urine microscopy

Urine samples were examined for the presence of *S.haematobium* eggs as in sedimentation method of Cheesbrough (2006) [21]. Each urine sample was mixed thoroughly with a glass rod and three samples were taken each 10 ml urine, one sample for sedimentation centrifugation , other 10 ml urine sample for Nuclepore membrane filteration technique and the third sample for circulating antigen in urine detection.The first 10 ml transferred into centrifuge tube and centrifuged at 2000 rpm for 5 minutes at room temperature. The supernatant was then discarded and sediment transferred to a microscope glass slide and covered with a cover slip. A drop of Lugol's Iodine was added onto the cover slip prior to examination. Examination of the entire sediment was carried out using x10 objective of a compound light microscope.

The second 10 ml urine sample were examined using the Nuclepore membrane filteration technique for *S.haematobium* eggs detection as in method of Cheesbrough (2009) [22]. The third urine sample were examined using commercially circulating antigen kit according to the assay procedures.

2.5. Assay procedure :

For 100 urine samples, were tested for the presence of circulating antigen by a commercially available kit (Schistofast ABC Diagnostic, New Damietta city ARE). Which were the same kit used by medical laboratories in Egypt), these steps according to company mehod.

Note :1- the urine sample was centrifuged (2000 rpm for 5 min.) to remove any turbidity e.g.pus cells, RBCs, -----etc. If centrifuge was not available, urine was leaved for about 30 min. supernatant was used.

2- To test the positive and the negative controls(precoated Devices), the assay procedure was followed up except step no. 1 (the addition of urine sample).

* the membrane surface of the test cartilage washed by adding 3 drops of solution(A)(wash solution), drops were allowed for complete absorption.

1- Three drops or (200 ul) of urine was added (supernatant),using new plastic pipette/sample

2- solution (B),blocking reagent, shaked gently, 2 drops were added, drops were allowed to be completely absorbed

3- solution (C),specific MoAb solution, shaked gently, 2 drops were added, drops were allowed to be completely absorbed

*Washed by addition of 3 drops of solution (A), the drops allowed for completely absorbed .

4- solution (D),alkaline phosplate conjugate ,shaked gently, 2 drops was added, drops allowed to be completely absorbed.

* Washed by addition of 3 drops of solution (A), the drops allowed for complete absorption .

5- Two drops or (100 ul) of solution (E), substrate solution, were added using new plastic pipette, and waited for 2 minutes .

6- Two drops of solution(F),stopper solution, were added, allowed to be completely absorbed, and the result was taken.

Result and report :

No colour ---------- Negative bilharzial antigen .

Violet colour ----------Positive bilharzial antigen .

Note : the violet colour intensity may be weak or strong according to the concentration of the bilharzial antigen in the urine sample .

2.6. Stool microscopy :

Stool samples were examined for the presence of *S.mansoni* eggs, only negative stool samples for *S.mansoni* eggs of 100 population study samples were taken for that research study.

Results collected, coded, tabulated and analyzed through computer facilities using statistical methods *S.haematobium* infection was defined as any number of eggs greater than zero found in 10 ml of urine, was performed to compared with circulating antigen by commercially kit for Diagnosis of *Schistosoma haematobium* Infection. data analysis Correlation of the circulating antigen results with the gold-standard parasitological data was done using diagnostic accuracy tests. Sensitivity, specificity, positive and negative predictive values (PPV and NPV), likelihood ratios for positive results (LR+) and diagnostic efficiency and the area under the curve (AUC) were calculated. In this study we considered using the sum of Nuclepore membraneas filteration technique and Centrifugation sedimentation technique results as a gold standard to compare them with circulating antigen by commercially kit [(23)].

3. Results

Table (1): shows diagnostic performance of circulating antigen in urine(CAg) by a commercially available kit (Schistofast ABC Diagnostic) as a diagnostic method for *Schistosoma haematobium* infection compared to microscopic examination techniques as the gold standard. In this study we considered using the sum of Nuclepore membraneas filteration technique and Centrifugation sedimentation technique results as a gold standard[(23)].

The results showed that circulating antigen in urine(CAg) by a commercially available kit had a sensitivity of 56% and specificity of 76%. PPVs was 70% whereas NPVs was 63.3%. As for diagnostic efficiency, it was (66%), where the area under the curve (AUC) was sufficient (0.6).

Table (1): Shows percentage of *S.haematobium* infection using a circulating antigen in urine(CAg) as a diagnostic method for *schistosoma haematobium* compared to microscopic examination techniques as the gold standard.

Test		**Microscopic techniques**		**Total No.**	**Sensitivity %**	**Specificity %**	**PPV %**	**NPV %**	**PLR %**	**NLR %**	Diagnostic Efficiency %	AUC
		Negative No .	Positive No .									
Circulating antigen in urine (CAg)	No	38	22	60	56	76	70	63.3	2.33	0.58	66	0.63
	Yes	12	28	40								
	Total	50	50	100								

4. Discussion

Detection of circulating schistosome antigens secreted by live schistosomes in body fluids with specific monoclonal antibodies (MAbs) has been shown to be promising approach to the detection of active infection and to the assessment of treatment efficacy and effectiveness of future vaccines [24,25] .

During schistosome infection, many of the eggs laid by the female worms become trapped in the tissues. The liver is particularly affected in *S. mansoni* and *S. joponicum* infections, while the bladder and ureters are the main organs of egg deposition by *S. haematobium* worms. As the major factor in the pathogenesis of schistosomiasis is the host granulomatous response to antigens secreted from the trapped eggs in host tissues (Pearce, 2005)[26]. Furthermore, early diagnosis is not possible because eggs are not found in feces and urine until flukes reach maturity (Armour et al., 1997) [27] . Therefore, several Immunodiagnostic methods have been developed for the diagnosis of light infections.

Years ago this commercial kit which used in this study also used in all medical laboratories in Egypt for detection of schistosoma antigen in urine is called schisto-fast bilharzial antigen in urine manufactured by ABC diagnostic, Damietta, Egypt. This showed that the sensitivity of circulating antigen in urine was 56%, where the specificity was 76% and positive predictive value 70% and kappa coefficient was 0.32 (fair), As for diagnostic efficiency it was (66%), where the area under the curve (AUC) was sufficient (0.6), while many of the assays based on antigen detection displays both high specificities and high sensitivities[1].

Zienab A, et al., (1995) detected circulating Schistosome antigen (CSA) in 97% of urine samples of *S. mansoni* infected school children, CSA was detectable in 100% of urine samples of mixed *S. mansoni* and *S.haematobium* infected patients. They also found specificity of the CSA reached to 98% [28] .

Ndhlovu P, et al., (1996) evaluated circulating anodic antigen (CAA) levels in different age groups in a Zimbabwean between rural community endemic for *schistosoma haematobium*. They found that specificity was 100% and the overall sensitivity was 97%.[29]

Mahfouz A, et al., (2012) evaluated different immunological techniques for diagnosis of *schistosoma haematobium* in Egypt through detecting of soluble egg antigen (SEA) in urine by different methods (latex agglutination technique LAT, sandwich ELISA and dot-ELISA) They found the sensitivity of circulating antigen in urine

ranged from 88.66% to 94.66%, while the specificity ranged from 91.25% to 96.25% [30] .

Circulating Schistosome antigen(CSA) disappear rapidly after treatment and can therefore be used for assessment of cure. However, the sensitivity of antigen detection varies from 55% to 100%, being low in low endemic areas with no advantage over stool and urine examination [1] .

Conflict of interest

There are no conflicts of interest.

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References :

1. Van Lieshout, L., A. M. Polderman, and A. M. Deelder**.** 2000. Immunodiagnosis of schistosomiasis by determination of the circulating antigens CAA and CCA, in particular in individuals with recent or light infections. Acta Trop. **77:**69–80.
2. Salah, F., A. El Bassiouny, I. Rabie, Z. Demerdash, M. Roshdy and Z. Shaker, 2006. Human *schistosomiasis haematobium*: Effective diagnosis of active infection using a pair of monoclonal antibodies against soluble egg antigen. Parasitol. Res., 99: 528-533.
3. Deelder AM, Klappe HT, van den Aardweg GJ, van Meerbeke EH (1976) *Schistosoma mansoni*: demonstration of two circulating antigens in infected hamsters. Exp Parasitol 40: 189-197.
4. Al-Sherbiny MM, Osman AM, Hancock K, Deelder AM, Tsang VC (1999) Application of immunodiagnostic assays: detection of antibodies and circulating antigens in human schistosomiasis and correlation with clinical findings. Am J Trop Med Hyg 60: 960-966.
5. Deelder AM, Qian ZL, Kremsner PG, et al. Quantitative diagnosis of schistosoma infections by measurement of circulating antigens in serum and urine. *Trop Geogr Med* 1994; **46:** 233–38.
6. McManus, D.P. and A. Loukas, 2008. Current status of vaccines for schistosomiasis. Clin. Microbiol. Rev., 21: 225-242.
7. Doenhoff MJ, Chiodini PL, Hamilton JV. [Specific and sensitive diagnosis of schistosome infection: can it be done with antibodies?]. *Trends in Parasitology* 2004;**20**(1):35–9.
8. Cavalcanti MG, Silva LF, Peralta RH, Barreto MG, Peralta JM. [Schistosomiasis in areas of low endemicity: a new era in diagnosis]. *Trends in Parasitology* 2013;**29**(2):75–82.
9. Rollinson D, Knopp S, Levitz S, Stothard JR, Tchuem Tchuente LA, Garba A, et al. [Time to set the agenda for schistosomiasis elimination]. *Acta Tropica* 2013;**128**(2): 423–40.
10. Lambertucci JR. Acute schistosomiasis mansoni: revisited and reconsidered. Mem Inst Oswaldo Cruz. 2010;105(4):422-35.1 Ferreira FT et al. POC-CCA for diagnosing schistosomiasis**364**
11. Lambertucci JR, Drummond SC, Voieta I, Queiróz LC, Pereira PPN, Chaves BA, et al. An outbreak of acute *Schistosoma mansoni* schistosomiasis in a non-endemic area of Brazil: A report on 50 cases, including five with severe clinical manifestations. Clin Infect Dis. 2013;57(1):e1-6.
12. DeJonge N, Decaluw P, Hilberath GW, Krijger FW, Polderman AM, Deelder AM, Circulating anodic antigen levels in serum before and after chemotherapy with praziquantel in schistosomiasis mansoni. Trans R Soc Trop Med Hyg 1989; 83: 368-372.
13. Van Leishout L, Dejonge N, Mansour MM, Bassily S, Krijger FW, Deelder AM. Circulating cathodic antigen levels in serum and urine of schistosomiasis patients before and after chemotherapy with prazequantel. Trans R Soc Trop Med Hyg 1993; 87: 311-312.
14. Feldmeier H, Nogueira-queroz JA, Peixoto-queiroz MA, Doehring E, Dessant JP, De

Alencar JE, et al. Detection and quantification of circulating antigen in schistosomiasis by monoclonal antibody. the quantification of circulating antigens in human schistosomiasis mansoni and haematobium: relation to intensity of infection and disease status. Clin Exp Immunol 1986; 65: 232-243.

15. WHO (1998): Report of the WHO informal consultation on schistosomiasis control, Geneva WHO/ CDS/ CPC/ S1P/ 1999. 2.
16. Ahmed A. Yameny. Validity of questionnaire to identify *Schistosoma haematobium* infection. J Biosci App Res .2017;3(4): 221-228.
17. Ahmed A. Yameny. The Validity of Haematuria and Proteinuria by chemical reagent strip for diagnosis of *Schistosoma haematobium* infection. J Biosci App Res .2017;3(4): 229- 236.
18. WHO. 2007. Report of inter country meeting on strategy to eliminate schistosomiasis from the Eastern Mediterranean Region. Muscat, Oman.6-8 November.
19. Ahmed A. Yameny. The evolving schistosomiasis agenda 2017- 2020 in Egypt : Moving from control toward final elimination. J Biosci App Res .2017;3(2): 48- 54.
20. Eyong ME, Ikepeme EE, Ekanem EE. Relationship between *Schistosoma haematobium* infection and urinary tract infection among children in South Eastern, Nigeria. Niger Postgrad Med J2008;15:89‑93.
21. Cheesbrough M. District laboratory practice in Tropical countries part 2. United Kingdom :Cambridge University Press. Cambridge. 2006; 323-41.
22. Cheesbrough M. Laboratory-practice-in-tropical-countries-part-1. United Kingdom :Cambridge University Press. Cambridge. 2009;p.357
23. Ahmed A. Yameny. Evaluation of Nuclepore Filteration Technique for Diagnosis of *Schistosoma haematobium* Infection.J Biosci App Res. 2015;1(6):335-341.
24. El-Morshedy H., Kinosien B., Barakat R., Omer E ., Khamis N., Deelder A. M., et al. (1996) Circulating anodic antigen for detection of *Schistosoma mansoni* infection in Egyptian patients. Am. J. Trop. Med. Hyg. **54**:149–153.
25. Polman K., Stelma F. F., Gryseels B., Van Dam G. J., Talla I., Niang M., et al. (1995) Epidemiological application of circulating antigen detection in a recent *Schistosoma mansoni*focus in north Senegal. Am. J. Trop. Med. Hyg. **53**:152–157.
26. Pearce, E.J., 2005. Priming of the immune response by schistosome egg. Parasite Immunol., 27: 265-270.
27. Armour, J., G.M. Urqhart and J.L. Ductan, 1997. Veterinary Parasitology. 2nd Edn., Blackwell Science Ltd., Oxford, New York, Pages: 64.
28. Demerdash ZA, Mohamed SH,Shaker ZA, Hassan SI, El attar GM, Saadel din AA, et al.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(2) 1995; 471 – 484.
29. Ndhlovu P,Cadman H,Gundersen S,Vennervald BJ,Friis H, Christensen NO.et al Circulating Anodic Antigen (CAA) levels in different age groups in a Zimbabwean rural community endemic for *schistosoma haematobium* determined using the magnetic beads Antigen- capture Enzyme –Linked Immunoassay. Am J Trop Med Hyg . 54(5),1996, pp. 537- 542 .
30. Mahfouz A., Mahana N., Rabee I.and El Amir A., 2012. Evaluation of Different Immunological Techniques for Diagnosis of*Schistosomiasis haematobium* in Egypt. *Biotechnology, 11: 10-19.*