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The Validity of Haematuria and Proteinuria by chemical reagent strip for diagnosis of *Schistosoma haematobium* infection

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ABSTRACT

Background : Urinary schistosomiasis is a chronic water-borne disease that affect the life of millions of people globally especially the rural areas of developing countries, Microscopic examination can quantify the intensity of the *S.haematobium* infection, however it is relatively insensitive especially in situations involving low level infections. Hematuria and proteinuria have been considered as alternatives for microscopic testing of urine to diagnose *S.haematobium* infection, and as a single tool in large-scale control programs. **Subjects and methods:** This study is a cross-sectional study designed to evaluate haematuria and proteinuria by chemical reagent strip for determine urinary schistosomiasis in the study population, to overall sample was 1000 patients of different ages 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 haematuria by chemical reagent strips had a sensitivity of 27.8% and specificity of 63.4%. When proteinuria was considered, sensitivity increased to 67.1% also specificity increased to 80.7%. When both haematuria and proteinuria was considered, sensitivity was 45.2% and specificity 85.9%. sensitivity in between the previous results. PPVs ranged between 6.13% and 22.9% whereas NPVs ranged between 91.1% and 96.6%. As for diagnostic efficiency, it was best for both haematuria and proteinuria (83.1%) followed by proteinuria 79.6% and least for haematuria (60.7%), where the area under the curve (AUC) was sufficient for both haematuria and proteinuria 0.61 and good for proteinuria(0.71),where it was not useful for haematuria (0.48). **Conclusion and Recommendations:** Most previous studies showed blood in urine or microhaematuria is more important than protein and used chemical reagent strips to detect microhaematuria and not protein. While this study showed detect of protein by chemical reagent strip is more important than microhaematuria by chemical reagent strip, and statistical parameters tested were higher with proteinuria than haematuria .The sensitivity, specificity, positive and negative predictive value, likelihood ratio for positive results, diagnostic efficiency and area under the curve all were higher in proteinuria than haematuria. The sum of proteinuria and haematuria had more specificity . In this study we demonstrate that the use of urine reagent strips for both proteinuria and haematuria might be considered for the diagnosis *of S. haematobiu*m where microscopy is unavailable.

Keywords: *Schistosoma haematobium***,** chemical reagent strip, proteinuria, haematuria, validity, urinary schistosomiasis.

1.Introduction

Urinary schistosomiasis is a chronic water-borne disease that affect the life of millions of people globally especially the rural areas of developing countries[1] (Houmsou *et al.*, 2012). Urinary schistosomiasis was reported in Upper Egypt with prevalence of infection ranging from 4.8 % in Qena to 13.7 % in Fayoum (Abdel-Wahab et al., 2000; El-Khoby et al., 2000). In 2016, due to different control measures, the overall prevalence fell down to less than 0.2% , but in 300 villages the prevalence still more than 3% , so Egypt has started of a campaign to reach the final elimination of schistosomiasis by 2020 [4]. Schistosomiasis has been eliminated in Iran, Lebanon, Morocco and Tunisia with absence of new recorded cases in the past few years (WHO, 2007)[5]. The control program in Egypt for Schistosomiasis elimination should be done upon to health education on risk factors with snail control and chemotherapy for infected patients[6].

Microscopic examination can quantify the intensity of the *S.haematobium* infection, however it is relatively insensitive especially in situations involving low level infections[7]. Alternative approaches to the parasitologic diagnosis have been tried with some success . One approach comprises assessment of morbidity, either by ultrasonographic detection of pathologic changes in *S.haematobium* (bladder) or by chemical reagent strip detection of blood and protein in urine during *S.haematobium* infection[8,9] . Both methods have shown great promise but the need for expensive equipment and comprehensive expertise present a major draw back in the use of ultrasonography in community-based diagnosis of human schistosomiasis [10] . Hematuria and proteinuria have been considered as alternatives for microscopic testing of urine to diagnose *S.haematobium* infection, and as a single tool in large-scale control programs[11]. The presence of reagent strip-detected hematuria and proteinuria has been reported to have high sensitivity and specificity for detecting *S.haematobium* when used to screen populations in endemic areas, and to evaluate response to praziquantil therapy [12] .

Haematuria is an important sign of urinary schistosomiasis in endemic areas. Macro –and micro-haematuria may be detected by visual observation or with the aid of a reagent strip [12]. Haematuria (HU), Leukocyturia(LU) and Proteinuria (PU) are widely accepted as indirect markers for urinary schistosomiasis in screening procedures [13] . Combined semiquantitative data of reagent strip determinations of proteinuria , haematuria and leukocyturia , the reagent strip index (RSI) , provide a more direct measure of acute pathology within the urinary tract [14] . These pathologic urine test strip findings correlate with the intensity of infection ; however ,they are not specific for *S.haematobium* [15] . Chemical reagent strip detection of blood in urine is anticipated to play a central role in community diagnosis of urinary schistosomiasis, although it is not useful for diagnosis of other *Schistosome* spp. infections [16] . However, the presence of blood in urine due to menstruation or genitourinary infections are factors that can confound urine reagent strip results [17] . Regarding causes of haematuria (RBCs in urine),it may be found in urinary schistosomiasis,genitourinary bacterial infections , acute glomerulonephritis, calculi in the urinary tract, malignancy of the urinary tract, sickle cell disease , leptospirosis, infective endocarditis and haemorrhagic conditions [18].

Concerning proteinuria , its degree reflects the severity of the glomerular or tubular urinary disease. It should always be considered to indicate underlying disease until proved otherwise also it helps in assessing prognosis and response to treatment[19]. The use of sensitivity and specificity of diagnostic tests does not provide the most practical information.Predictive values and likelihood ratios are most useful for assessing the value of reagent strip-detected hematuria and proteinuria in predicting the presence or absence of infection within subgroups of the populations [20] . So in this study will calculate predictive values , likelihood ratios, diagnostic efficiency and area under the curve for more practical information. Quality control of reagent strip tests is fulfilled

through control reference solutions which are available from manufacturers for some strips.The performance of most strip tests can also be controlled by checking regularly the strip reactions against those obtained by standard chemical tests .Control urines of known negative and positive reactions should also be prepared and tested with patients specimens . Up –to-date manufacturers` literature must always be consulted regarding the quality control of different strips and the substances which may interfere with the various reactions [19] . This study used both chemical reagent strip and microscopic examination of urine samples to detect of *S.haematobium* eggs for 1000 patient samples to evaluate the validity of haematuria and proteinuria compared with microscopic examination for diagnosis of *S.haematobium* infection.

Research objective :

To evaluate the validity of haematuria and proteinuria by chemical reagent strip compared with microscopic examination for diagnosis of *S.haematobium* infection.

2. Material and methods

2.1. Study population and ethical consideration

This study included 1000 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 haematuria and proteinuria by chemical reagent strip for determine urinary schistosomiasis in the study population. This study was targeting customers who came for laboratories of health centers for urine analysis.

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[21]. In this study, urinary schistosomiasis was defined as the presence of ova of *S.haematobium* in the urine.

2.4. Urine microscopy

Urine examination was carried out in the laboratory of health centers and by the researcher. Urine samples were examined for the presence of *S.haematobium* eggs as in sedimentation method of Cheesbrough (2006) [22]. Each urine sample was mixed thoroughly with a glass rod and two samples were taken each 10 ml urine, one sample for sedimentation centrifugation and other 10 ml urine sample for Nuclepore membrane filteration technique.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) [23].

2.5. Chemical reagent strip:

For 1000 urine samples, a chemical reagent strips were tested for the presence of protein and blood,(Teco diagnostics Anaheim, CA, U.S.A. Which were the same strips used by health center laboratories).

-Blood: the test is based on the pseudoperoxidase action of hemoglobin and erythrocytes which catalysis the reaction of 3,3',5, 5'-tetramethyl-

benzidine and buffered organic peroxide. The resulting colors range from orange to yellow-green and dark green. Very high blood concentration may cause the color development to continue to dark blue.

-Protein : this test is based on the protein error-of-indicator principle. At a constant PH, the development of any green color is due to the presence of protein, colors range from yellow for a negative reaction to yellow-green and green to blue-green for a positive reaction .

Test procedure :
a-Remove from the bottle only enough strips for immediate use and replace cap tightly.
b-Completely immerse reagent areas of the strip in fresh, well-mixed urine. Remove the strip immediately to   avoid dissolving out the reagent areas.

c-While removing, touch the side of the strip against the rim of the urine container to remove excess urine, blot   the lengthwise edge of the strip on an absorbent paper towel to further remove excess urine and avoid   running over (contamination from adjacent reagent pads).

d-Compare each reagent area to its corresponding color blocks on the color chart and read at the times specified, proper read time is critical for optimal results.

e-Obtain results by direct color chart comparison.
  Reagent areas may be read between 1-2 minutes for screening positive urine from negative urine , changes of   color after 2 minutes are of no diagnostic value.

2.6.Statistical analysis

  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

haematuria and proteinuria by chemical reagent strip for Diagnosis of *Schistosoma haematobium* Infection. data analysis Correlation of the reagent strip 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 haematuria and proteinuria by chemical reagent strip [23].

3. Results

  Table (1): shows diagnostic performance of haematuria , proteinuria by chemical reagent strips and both as diagnostic methods for *Schistosoma haematobium* 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 haematuria by chemical reagent strips had a sensitivity of 27.8% and specificity of 63.4%. When proteinuria was considered, sensitivity increased to 67.1% also specificity increased to 80.7%. When both haematuria and proteinuria was considered, sensitivity was 45.2% and specificity 85.9%. sensitivity in between the previous results. PPVs ranged between 6.13% and 22.9% whereas NPVs ranged between 91.1% and 96.6%. As for diagnostic efficiency, it was best for both haematuria and proteinuria (83.1%) followed by proteinuria 79.6% and least for haematuria (60.7%), where the area under the curve (AUC) was sufficient for both haematuria and proteinuria 0.61 and good for proteinuria(0.71),where it was not useful for haematuria (0.48).

Table (1): Diagnosis of *S.haematobium* infection using chemical reagent strip compared to microscopic examination technique.

Test		Microscopic techniques		Total No.	Sensitivity %	Specificity %	PPV %	NPV %	PLR %	NLR %	Diagnostic Efficiency %	AUC
		Negative No .	Positive No .									
Haematuria by chemical reagent str1ip	No	585	57	642	27.8	63.4	6.13	91.1	0.76	1.14	60.7	0.48
	Yes	336	22	358								
	Total	921	79	1000								
Proteinuria by chemical reagent strip	No	743	26	769	67.1	80.7	22.9	96.6	3.47	0.41	79.6	0.71
	Yes	178	53	231								
	Total	921	79	1000								
Both Haematuria andProteinuria by chemical reagent strip	No	487	23	510	45.2	85.9	19.2	95.5	3.21	0.64	83.1	0.61
	Yes	80	19	99								
	Total	567	42	609								

PPV,Positive predictive value; NPP, Negative predictive value; PLR, Likelihood ratio for positive results; NPL,Likelihood ratio for negative results; AUC Area under the curve.

4. Discussion

Reagent strips for microhaematuria and simple questionnaires for red urine are easy ,cheap , and effective tools for the screening and rapid epidemiological assessment of urinary schistosomaisis.[25]. such indirect diagnostic methods are less satisfactory for schistosomiasis intestinal or hepatic disease [26] . Since the early 1980s, reagent strip testing for microhaematuria has been used as an indirect diagnostic assay for *Schistosoma haematobium* [9].

This study used the same chemical reagent strip which used in health center laboratories (Teco diagnostic USA), to detect blood and protein in urine, most or nearly all previous studies showed blood in urine or microhaematuria is more important than protein and used chemical reagent strips to detect microhaematuria and not protein. But this study showed detect microhaematuria by chemical reagent strip has a sensitivity of 27.8%, specificity of 63.4%, positive predictive value 6.13% and negative predictive value of 91.1%, while protein in urine by chemical reagent strip had a sensitivity of 67.1%, specificity of 80.7%, positive predictive value of 22.9% and negative predictive value of 96.6%, %), where the area under the curve (AUC) was sufficient for both haematuria and proteinuria 0.61 and good for proteinuria(0.71),where it was not useful for haematuria (0.48). By this study the detection of proteinuria by chemical reagent strip has a more sensitivity and more specificity than haematuria for diagnosis of *S.haematobium* infection.

Poygensee G et al (2000), reported the validity of urine reagent strip reading which haematuria was showed a sensitivity of 64.9%, specificity of 67.7%, PPV of 54.7% and NPV of 57.5%, but proteinuria showed a sensitivity of 12.2%, specificity of 91.3%, PPV of 37.9% and NPV of 75.3%[27].

Nagi MA,(2005), in Yamen evaluate the reagent strip, he showed that it was has a sensitivity of 56.5%, specificity of 57.3%, PPV of 72.2% and NPV of 39.9%[28].

Michael D et al,(2007), used microhaematuria with reagent strips as a rapid assessment for urinary Schistosomiasis it showed a sensitivity of 85%, specificity of 98.5%, PPV of 83.5% and NPV of 98.5%[29]. Sensitivity of these strips for detection of microhaematuria is higher than that of urine filtration (Michael D. French, et al., 2007; Robinson et al., 2009)[29,30]. Some authors have argued that most of this background microhaematuria is due to undetected *S.haematobium* explained by the lack of sensitivity of widely used diagnostic tools[31,32].

In 2015 Cochrane systematic review it has been summarised that reagent strip testing for *S. haematobium* diagnosis has an overall sensitivity and specificity of 75 % and 87 %, respectively [33]. where microhaematuria could not be associated with *S. haematobium* through reference microscopy [33,34].

A study in Nigeria(2017) recorded that: Proteinuria and haematuria were observed to occur in 67.0% and 79.0% respectively. Males had higher infections and higher occurrences of proteinuria and haematuria (72.5%, 88.2% respectively). There was no significant difference with respect to sex (P<0.05)[35].

5. Conclusion and Recommendations

Most previous studies showed blood in urine or microhaematuria is more important than protein and used chemical reagent strips to detect microhaematuria and not protein. While this study showed detect of protein by chemical reagent strip is more important than microhaematuria by chemical reagent strip, and statistical parameters tested were higher with proteinuria than haematuria .The sensitivity, specificity, positive and negative predictive value, likelihood ratio for positive results, diagnostic efficiency and area under the curve all were higher in proteinuria than haematuria. The sum of proteinuria and haematuria had more specificity .

these strips suitable for initial mapping and screening of urinary schistosomiasis in the field. However, microhaematuria and proteinuria are non-specific signs that could also result from other diseases such as urogenital infection, malignancy, immune system disorders, metabolic disorders and trauma. In field study, screening in high endemic area with schistosomiasis using of strip for detection of both proteinuria and haematuria is more valid than detection of haematuria alone .

In this study we demonstrate that the use of urine reagent strips for both proteinuria and haematuria might be considered for the diagnosis *of S. haematobiu*m where microscopy is unavailable.

Conflict of interest

There are no conflicts of interest.

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