Journal of the Egyptian Society of Parasitology, Vol.43, No.3, December 2013
J. Egypt. Soc. Parasitol., 43(3), 2013: 577 - 589

PRESENT SITUATION OF *SCHISTOSOMA HAEMATOBIUM* INFECTION AMONG PRIMARY SCHOOL AGED CHILDREN IN SOME AREAS OF QUALYOBIA GOVERNORATE-EGYPT

By

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Abstract

The current study determined the present situation of urinary schistosomiasis among primary school children in some areas of Qualyobia governorate in Egypt using different diagnostic methods, and to study the effect of *Schistosoma haematobium* infection on growth parameters of the affected children.The Results showed that The prevalence rate of *S. haematobium* infection among school children was 5.3% (32/600-child). The infection was more prevalent in males (7.3%) than females (3.1%). The mean age of children was 9.0±1.76. All infected children showed hindered growth parameter in comparison to corresponding children (low height, weight and body mass index (BMI) for age Z-score). Water contact activities were more frequent in males than females (P<0.001). The dipstick test specificity was 96.4% (versus 96.7% by microscopic examination) and the sensitivity was 88.2% (versus 76.5% by microscopic examination), which was statistically significant (P<0.001).

Key words: *Schistosoma haematobium*, Children's growth parameters, Circulating cathodic antigen.

Introduction

Bilharziasis is a parasitic disease dates back to the ancient times, as its eggs were identified in Egyptian mummies (1200 B.C.) and the main symptom, hematuria, was recorded in the oldest papyrus of Kahum, 1900 B.C. (Badr, 1981).

Urogenital schistosomiasis remains one of the most prevalent parasitic problems in the tropical and subtropical countries. *Schistosoma haematobium* is the most prevalent form of schistosomiasis in Africa and the Middle East affecting approximately 107 million people, where children carry the heaviest burden of infection (Midzi *et al*, 2008) as urogenital schistosomiasis causes haematuria, dysuria, nutritional deficiencies, impaired memory and cognition, growth retardation, decreased physical performance and anaemia (Jukes *et al*, 2002; Bhargava *et al*, 2003; Koukounari *et al*, 2007).

Deribew *et al.* (2013) reported higher anemia in infected children than non-infected ones with the hemoglobin level negatively correlated with the number of *S. haematobium* eggs

In the detection of parasitic infection, the traditional methods based on microscopy often have low sensitivity and/or specificity compared with the newer, molecular tests. Detection of parasite-specific DNA in urine has been demonstrated and this has similar specificity but improved sensitivity (Clive Shiff, 2012). WHO (2011) recommended that young children living in *Schistosome*-endemic areas should be considered for treatment with Praziquntel during child health campaigns at the standard dose of 40 mg/kg**.** The importance of identifying cases in early childhood is two-fold: firstly, to provide treatment and to arrest development of morbidity from infections acquired early in childhood, and secondly, tackling infections in this age-group might reduce local environmental contamination and putative *disease* transmission (Stothard *et al*, 2011).

This present work aimed to assess situation of urinary schistosomiasis among primary school children in some areas of Qualyobia Governorate using different diagnostic methods, and to study the effect of *S. haematobium* on growth parameters of these children.

Subjects, Materials and Methods

Ethics Statement: The study objectives were explained to school children and their parents for understanding and cooperation. The study protocol was approved by the ethical review board of the faculty of Medicine, Benha University.

This study was conducted in Benha City an urban area and Kafer El-Gazar village a rural one in Qualyobia Governorate. A total of 600 urine samples were collected from children aged 5-12 years attending ten primary schools in Benha city (540 samples) and a primary school in Kafer El-Gazar village (60 samples) from September 2011 to September 2012. Samples were examined in Department of Parasitology, Faculty of Medicine, Benha University.

The following was done for all children: 1-History was taken from each child; age, sex, grade, residence and symptoms of acute infection as; dysurea, frequency, gross haematuria (macrohaematuria) and suprapubic pain. 2- Clinical examination especially anthropometric measurements [body weight and height and body mass index (BMI)]. Also, to detect signs referred to liver affection as; hepatomegaly, splenomegaly, portal hypertension and ascites.

Collection of urine samples:_About 20ml of midstream urine samples were collected in 50ml capacity clean plastic container labeled with his/her name and date of collection. Samples were collected by children themselves, who were previously carefully instructed with illustration aids. Samples were obtained between 10.00 am and 2.00 pm. Samples with visible haematuria were noted. The specimens were placed in a cold box with ice packs, immediately after collection. They were processed 1-2hrs of collection. In

situations where delay in transportation of specimens to laboratory was inevitable, ordinary household bleach was added to the urine samples (ratio; 1ml bleach: 50ml urine) to preserve any *Schistosome* ova present (Cheesbrough, 1998).

Each sample was examined by: a- Microscopic examination to detect *Schistosoma haematobium* ova (Baker et al, 1985). Ten ml of the urine was centrifuged at 1500 r.p.m for 5 minutes the supernatant was discarded to leave sediment which was transferred to the centre of a clean grease-free glass slide to which was added a cover slip. This was mounted on a light microscope and examined under both low power & high power objective lenses to identify *Schistosoma haematobium* ovum which is described as golden yellowish and elliptical in shape with a terminal spine.

b- Filtration of urine (Houmsou et al., 2011): Ten ml of urine was taken and filtered through an 8-um polycarbonate membrane in a filter holder with the help of a forceps. The filter holder was placed on a slid. A drop of lugol's Iodine was added and the slide was examined under microscope using (10x) & (40x) objective lenses. The number of eggs was counted per 10 ml of urine and intensities of infection were classified as 1-10 eggs, 11-49 eggs and >50eggs for light, moderate and heavy infections respectively.

C-Examination for microhaematuria (Mott *et al*, 1985): A Self-Stik^{R} reagent strip (ChungDo.Pharm.Co.Ltd.Seoul, Korea) was carefully dipped into the bottle containing urine for 5 seconds. The resulting change in colour of the strip was compared with manufacturer's colour chart to estimate the amount of blood in the urine.

d- Circulating cathodic antigen (CCA) urine dipstick test (Ashton et al., 2011): CCA tests (Rapid Medical Diagnostics; Pretoria, South Africa) were conducted according to the manufacturer's instructions on single urine samples collected from 96 pupils who had positive history of water activities. CCA results were graded according to test band strength, where weak positive was defined by the control band being darker than test band, while a strong positive was defined by a test band darker or the same colour as the control band.

Anthropometric measurements: Anthropometric measurements were conducted for body weight, height and body mass index (BMI) using Z-score. Body weight was measured with minimum clothing to the nearest 100g with minimum clothing (only T-shirts and shorts) and using battery-operated digital scales. For height, the child stood erect against a wall and the height measured with a flexible tape and recorded to 0.1 cm. BMI (a measure of weight relative to height) as expressed as (kg/m2).

Statistical analysis: Data were tabulated and analyzed using SPSS version 16 software (Spss Inc, Chicago, ILL Company). Categorical data were presented as number and percentages while quantitative data were expressed as mean and standard deviation. Chi square test (X^2), Fisher's exact test and student "t" tests were used as tests of

significance. Kappa test was used to assess the agreement degree between microscopic and serological methods. The accepted level of significance in this work was stated at 0.05 (P <0.05 was considered significant).

Results

The prevalence of *S. haematobium* infection among primary school children was (5.3%). (figure3). There was statistically significant difference between prevalence of *S. haematobium* infection in Benha city (4.3%) and Kafer-El-Gazar village (15.0%). Infection was more prevalent in age ranged from 9to10 years; the mean age of infected pupils was 9.0± 1.76. Boys had a significantly (*P*<0.022) higher infection rate (7.3%) than girls (3.1%).

The intensity of infection was higher (>50 eggs/10ml urine) in pupils attending urban schools (21.7%) than those attending rural one (11.1%) this was statistically significant (P=0.001*). There was highly statistical significant difference (*P*<0.002) between prevalence of *Schistosoma haematobium* infection in urban (4.3%) and rural (5.3%) areas. Water contact activities were more frequent in males than females. It was also higher in pupils with age ranged from 7 to 10 years old (P<0.001).

The symptoms and signs revealed upon questionnaire and examination by the pediatrician showed variable clinical presentations of the studied pupils. The more common symptoms in patients was urgency (6.3%) followed by dysuria (5.7%), suprapubic pain (25%) and gross haematuria from history (2.2%). All examined pupils were free from hepatomegaly or splenomegaly and none of them had taken any treatment for schistosomiasis. Reagent strip-detected microhematuria was 4.6% of the studied group.

There was statistically significant difference between mean weight of infected (22.9± 4.69 kg) and healthy pupils (22.8±8.03 kg) and also significant difference between mean height (121.9±4.51cm) of infected and healthy pupils (128.8±7.33cm) (*P*<0.001). There was low weight for age, height for age, BMI for age Z-score in positive children for *Schistosoma haematobium* eggs.

Four samples were microscopically negative for *S. haematobium* eggs but positive for (CCA) in urine. The agreement between the microscopic examination and detection of Circulating Cathodic *Schistosoma* adult antigen in urine using dipstick test was 95.8% (P=0.044*), comparing predictively of microscopic examination and (CCA) in diagnosis. The specificity of the dipstick test was 96.4% versus 96.7% for the microscopy and sensitivity was 88.2% versus 76.5% for the microscopy, with statistical significant, P<0.001.

Details are given in tables and figures.

Table 1: Questionnaire for *S. haematobium* among children

Variable		No.	%
Gender	Male	313	52.2
	Female	287	47.8
Residence	Urban	540	90.0
	Rural	60	10.0
School grade	1	100	16.7
	2	100	16.7
	3	100	16.7
	4	100	16.7
	5	100	16.7
	6	100	16.7
Water contact	No	504	84.0
	Yes	96	16.0
Past History of Schistosomiasis	No	600	100.0
Prior treatment	No	600	100.0
	Mean ± SD	Minimum	Maximum
Age (years)	8.7±1.8	6	12
Weight (kg)	27.6±8.0	14	56
Height (cm)	128.4±7.4	107	151

Table 2: Age and sex distribution of children that had water contact activities

Age (years)	Females (n=287)		Males (n=313)	
	Positive	Negative	Positive	Negative
>6	2	82	5	51
7-8	10	60	20	71
9 -10	16	45	33	48
11-12	3	69	7	78
Total	31	256	65	248

X^2=11.1, P=0.001*

Table3: Prevalence of *S. haematobium* infection in relation to age of children.

Age	Number examined		Number of infected			
≤ 6	100		1 (0.01%)			
7-8	143		12 (8.39%)			
9-10	152		15 (9.86%)			
11-12	205		4 (1.95%)			
Total	600		32 (5.3%)			
Variable	No eggs in urine (N=568)		Positive eggs in urine (N=32)		St. 't'	P
	Mean	± SD	Mean	± SD		
Age	8.65	1.84	9.0	1.76	0.95	0.34

Table 4: Relation between locality and intensity of infection

Intensity of infection	Urban area	Rural area
Low (1-10 eggs/10ml urine)	8 (34.8%)	6 (66.7%)
Moderate (11-49 eggs/10ml urine)	10 (43.5%)	2 (22.2%)
High (>50 eggs/10ml urine)	5 (21.7%)	1 (11.1%)
Total	23 (100%)	9 (100%)

Fisher's Exact P=0.001*

Table 5: Prevalence of *S. haematobium* according to residence

Residence		Positive	Total
urban	Count	23	540
	% within residence	4.3%	100.0%
rural	Count	9	60
	% within residence	15.0%	100.0%
Total	Count	32	600
	% within residence	5.3%	100.0%

Fisher's Exact P =0.002*

Table 6: Clinical findings among children

Clinical findings	Result	No.	% (100.0)
Urgency	+ve	38	6.3
Dysuria	+ve	34	5.7
Frequency	+ve	36	6.0
Gross haematuria	+ve	13	2.2
Suprapubic pain	+ve	15	2.5
Suprapubic tenderness	-ve	0	-
Hepatomegaly	-ve	0	-
Splenomegaly	-ve	0	-

Table 7: Growth parameters of children as measured by Z score.

Variable	*S.h.* egg	No.	Mean	Std. Deviation	95% CI of the mean		St. "t"	P
					Lower	Upper		
Z score(weight)	positive	32	-0.588	0.589	-0.8	-0.38	3.45	0.001*
	negative	568	0.033	1.008	-0.05	0.116		
Z score(height)	positive	32	-0.883	0.611	-1.1	-0.66	5.25	<0.001*
	negative	568	0.049	0.994	-0.32	0.132		
Z score(BMI)	positive	32	-0.334	0.723	-0.59	-0.07	1.95	0.052
	negative	568	0.019	1.010	-0.064	0.102		

Table 8: Agreement between microscopic examination and CCA detection

(CCA) for *Schistosoma haematobium*		*S. h.* negative	*S. h.* positive	Total
Negative	Count	60	Zero	60
	% within *S. h.* egg	93.8%	0.0%	62.5%
positive	Count	4	32	36
	% within *S. h.* egg	6.2%	100.0%	37.5%
Total	Count	64	32	96
	% within *S. h.* egg	100.0%	100.0%	100.0%

Kappa test =0.91 P=0.044* Degree of agreement=95.8%

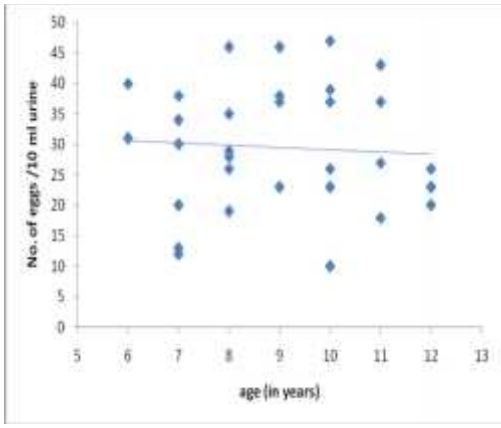

Fig. 1: Age and intensity of infection

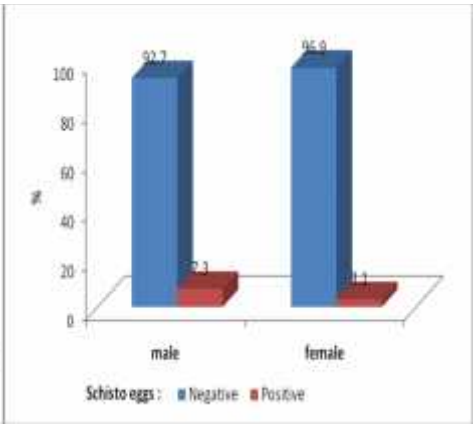

Fig.2: *S. haematobium* in relation to sex.

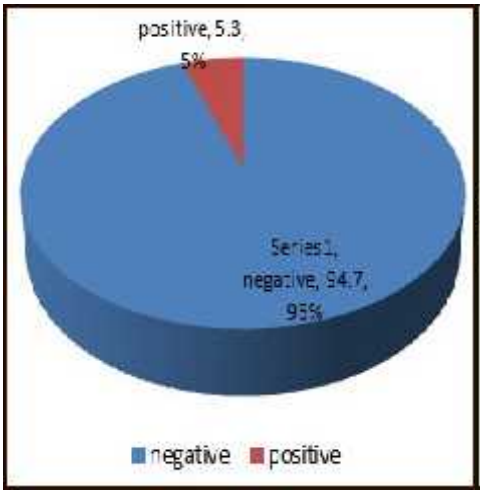

Fig. 3: *S. hematobium* by microscopy

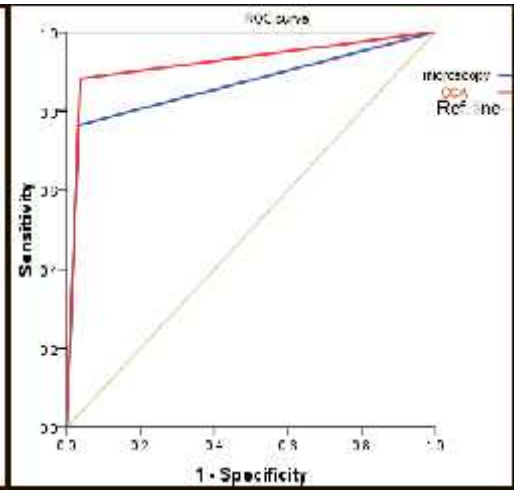

Fig. 4: Validity and predictivity by microscopy & CCA.

Discussion

The present study showed that *S. haematobium* is still actively transmitted in Qualyobia Governorate. The overall prevalence of infection among primary school aged children is higher (5.3%) than that previously reported 0.08% (El-Khoby *et al*, 2000). This may be due to habits of the population specially children to use water canals in daily activities and recreational purposes, also, lack of compliance to effective oral antibilharzial drugs.

In Egypt, the first country-wide survey, published by Scott (1937) reported high prevalence rates of *S. haematobium* infection (60% in Nile Delta, 89% in Fayoum and 25% in Upper Egypt) while, Miller (1976) reported a decrease in prevalence (30% in Nile Delta, 27% in Beni-Suef, and 6% in Upper Egypt). Barakat *et al*. (1998) reported that the prevalence of human schistosomiasis in Egypt declined due to control programs over the last decade.

In the present study, intensity of infection increased with age, which agreed with Midzia *et al*. (2009) who reported that intensity of infection increased of age. In the present study, intensity of infection showed significantly higher level in Benha City versus Kafer El-Gazar village, low intensity of schistosomiasis among school children in Kafer El-Gazar village that may be attributed to improved sanitation or provision of safe water. All houses have piped water that reduced water contact activities for domestic purposes. This agreed with Barakat *et al*. (2000) who reported low intensity of infection relative was accompanied with the Egyptian hygienic progress of community.

In the present study, males were significantly more infected than the females. This agreed with El-Khoby *et al.* (2000) in Egypt who reported that males had higher infection rates and ova counts than females in all age groups. Rine *et al.* (2013) in Nigeria reported that infections were high in males 23 (18.70%) than in females 3(8.1%) students. The reason for the higher prevalence among the male children is presumably due to higher water contact activities by male pupils especially in swimming and bathing in cercariae-infested water bodies. In addition, females are generally restricted from these on religious and socio-cultural grounds

In the current study, there was a strong relationship between *S. haematobium* infection and the children's nutritional status as expressed by the anthropometric measures which used to assess the nutritional status of children in relation to urinary schistosomiasis. Besides, anthropometry was described as the single most universally applicable, inexpensive, and non-invasive method available to assess the size, proportions, and composition of the human body. In the nutrition field, decrease height and/or weight relative to reference data have been used as classic indicators of under nutrition for individuals and groups (WHO, 1995). Nutritional status indicators were classified and standardized into sex-specific Z-scores for height-for-age

(HAZ), weight-for-height (WHZ) and weight-for-age (WAZ) these reference curves recommended for international use (Hamill et al, 1979; WHO, 1986). Children were classified as stunted, wasted, and underweight if their HAZ, WHZ and WAZ was < - 2 SD respectively. In the current study, children infected with *S. haematobium* had significantly lower body weight, height and BMI than their mates (P<0.001) (low Z-score for age). This could be explained on the fact that parasitic infections such as schistosomiasis and soil transmitted helminthes infections cause anorexia and poor absorption of nutrients.

Stephenson *et al*. (1985) in Kenya reported that relatively heavy infections of *S. haematobium* were the cause iron loss, which produced iron deficiency anemia and reduced physical fitness of children. Nduka et al., (2006) reported that both stunting (low height-for-age) and underweight (low weight-for-age), in comparison with healthy reference populations, can be used as indicators of malnutrition. Stunting, in particular, is thought to be a good indicator of malnutrition and often represents a state of chronic nutritional stress. Terer *et al.* (2013) conducted a study for evaluation of the Health-related Quality of Life (HrQoL.) of children in *S. haematobium*-endemic communities in Kenya. They conclude that exposure to urogenital schistosomiasis is associated with a 2–4% reduction in HrQoL. On the contrary Keanu *et al.* (1994) in Nigeria evidenced that there was no significant impact of urinary schistosomiasis regarding anthropometric parameters among the infected children compared to controls, although this was attributed to low intensity of the infection among the children investigated.

In the present work, egg detection based on the microscopy was the primary gold standard in diagnosis of *S. haematobium*. The degree of agreement between microscopy and CCA dipstick test was 95.8% (Kappa test =0.91 & P=0.044*). Specificity of CCA urine dipstick test was 96.4% and sensitivity was 88.2%, with statistical significance (P<0.001). Obeng *et al.* (2008) recorded that CCA urine dipstick test gave low sensitivity (41%) and high specificity (91%). Ruth *et al*. (2011) reported that the diagnostic accuracy of CCA urine dipstick test was poor in detecting *S. haematobium* infections, with a sensitivity of 36.8% and specificity of 78.9%. Artemis *et al*. (2009) reported that urine antigen detection test showed similar sensitivity to microscopy.

The present results contrasted with Stothard *et al*. (2006) who failed to detect CCA positivity among study groups living in endemic communities in Zanzibar, Niger and Burkina Faso, and did not detect infection among patients with heavy *S. haematobium* infection.

In the present work, an interesting observation was that within the group of zero egg counts, four children were positive for CCA and microhematuria. This emphasizes that one urine sample examined may not be the best indicator of infection status. This agreed with De Clercq *et al*. (1997) and Berhe *et al*. (2004) who suggested that daily fluctu-

ation in egg excretion might be the cause. Besides, Deelder *et al*. (1994); Stothard *et al*. (2006) and Ayele *et al.* (2008) reported CCA positive while, egg was negative cases. However, in the present study, differences in sensitivity were observed. The discrepancy between CCA and other parameters depended on infection intensity, and the haematuria dipsticks proved sufficiently sensitive and specific.

Conclusion

The outcome data showed that urinary schistosomiasis is actively transmitted among school children in Qualyobia Governorate. It is of vital importance for national control programs in Egypt to monitor the status of schistosomiasis in other urban cities in Qualyobia. All school children must be treated randomly as this infection affect their growth. Also, the circulating cathodic antigen (CCA) urine dipstick test is specific for the genus *Schistosoma* and fulfilled the requirements for a simple, rapid test, no expensive equipment, and performed with minimal training. No doubt, there is an urgent need for simple and reliable tests in endemic areas for the diagnosis and for chemotherapy the follow-up among schoolchildren.

Acknowledgments

The authors would like to acknowledge mangers of primary schools in the study area and would like to thank all Members of Parasitology laboratory, Benha Faculty of Medicine, for their kind help.

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