Journal of Natural and Medical Sciences (JNMS)
vol. **15** (2) 2014
ISSN 1858-6805
e-ISSN 1858-6813

SUST
Journal of Natural and Medical Sciences

Journal homepage: http://journals.sustech.edu/

Prevalence of *Schistosoma Mansoni* Using Different Diangnostic Techniques In The Gezira State, Sudan

Lana M. El-amin*[1], Ahmed Monis[2], Nagla Gasmelseed[3], Abakar A.D[1]

*1-Department of Medical Parasitology, Faculty of Medical Laboratory Sciences, Gezira University. E-mail:lana.alamin@gmail.com
2-Department of Oncology, Institute of Cancer, University of Gezira.
3-Department of Molecular Biology, Institute of Cancer, University of Gezira.

RECEIVED: 29/4/2014 ACCEPTED: 12/6/2014

ABSTRACT

The main objective of this study was to compare different diagnostic methods of *Schistosoma mansoni* in the Gezira State Sudan during (2005-2007). 438 pupils were selected with mean age 11.42 ± 2.588 (6-20 yrs). 192 (43.9%) were males and 246 (56.2%) were females. All the pupils were examined for *Schistosoma mansoni* using Kato-Katz technique. A questionnaire as a diagnostic tool was filled by each student. 203 were selected randomly to be examined by ultrasound (U/S). Fifty *S. mansoni* negative stool samples were examined by Polymerase Chain Reaction (PCR). The present study showed that prevalence of *S. mansoni* was 29.2% by Kato-Katz technique, 27.2% by questionnaire, 50.5% by ultra sonography (U/S), and 26% by PCR. The sensitivity and specificity of the questionnaire was 34.4% and 75.8% respectively (p=0.0256), with positive predictive value (PPV) equal to 37%. Ultrasound sensitivity was 54.4% and the specificity was 51.0% (P=0.591) with a PPV of 30%. The sensitivity of PCR was 100% while the specificity was 74% with a PPV 18% (p=0.023). The study revealed that questionnaire was a good test for screening and identification of the exposure and infection, but ultrasonography cannot be used as a diagnosing technique. PCR was considered the best diagnostic tool for confirmation of *S. mansoni* infection. The study recommended the use of the PCR for *S. mansoni* diagnosis and the possibility of using Questionnaire as screening tools for this parasite in a sustainable control program.

المستخلص

هدفت هذه الدراسة إلى مُقَارَنَة عدة طرق تشخيصية مختلفة لداء المنشقات في منطقة مستوطنة في قرية تقع في ولاية الجزيرة خلال الفترة من (2005–2007). تم إختيار 438 تلميذ وتلميذه بمدى عُمري بين (6–20سَنَة) و متوسط الاعمار 2.588 ± 11.42 . عينات البراز جُمعت مِنْ 438 تلميذ و تلميذة. طرق التشخيص تشمل: الإستبيان، إختبار الكاتو–كاتز، الأشعة فوق الصوتية وطريقة تفاعل الخميرة السلسلي التكاثري. مائتان و ثلاثة تلميذَ مدرسة اختيروا بشكل عشوائي مِنْ بين الـ 438 وفَحصوا بالأشعة فوق الصوتية. وخمسين عينة براز ذات نتيجة سلبية للمنشقة المانسونية لطريقة الكاتو–كاتز تم فحصها بطريقة تفاعل الخميرة السلسلي التكاثري.

كان معدل إنتشار المنشقة المانسونية 29.2 % بالطرقية المعيارية (الكاتو–كاتز) ، وكان بالإستبيان 27.2 % و50.0 % بالأشعة فوق السمعية و 26.0% بطريقة تفاعل الخميرة السلسلي التكاثري. الحساسية والخصوصية للإستبيان كان 34.4% و75.8% على التوالي (القيمة الإحتمالية =0.0256) و القيمة التنبؤية الإيجابية كانت

Journal of Natural and Medical Sciences (JNMS)
vol. **15** (2) 2014
ISSN 1858-6805
e-ISSN 1858-6813

37 %. الأشعة فوق الصوتية لتشخيص المنشقة المانسونية حسّاسيتها كَانتْ 54.4 %، وخصوصيها 51.0% (القيمة الإحتمالية =0.591) أما القيمة التنبؤية الإيجابية كانت 30 %، وكانت حسّاسيه طريقة تفاعل الخميرة السلسلي التكاثري 100% والخصوصية 74% والقيمة التنبؤية الإيجابية كانت 18% (القيمة الإحتمالية =0.023). كما كَشفتْ الدراسةَ بأنّ الإستبيان للمنشقة المانسونية يعتبر جيدُ لتشخيص وتعريفِ التعرّضِ للعدوى، أما الأشعة فوق الصوتية فلا يُمكن أنْ تستَعملَ في فحص تلاميذِ المدارس وكما تعتبر طريقة تفاعل الخميرة السلسلي التكاثري هي أفضل طريقة للتشخيصِ و التأكيد. الدراسة أوصتْ بأن تشخيص طريقة تفاعل الخميرة السلسلي التكاثري يُتطلّبُ كفحص تأكيدي للنَتائِجِ السلبية بالنسبة لطريقة الكاتو كاتز.

KEYWORDS: *S. mansoni, kato katz, US, PCR, Prevalence*

INTRODUCTION

Schistosomiasis is endemic in 74 countries worldwide over 650 million people globally are at risk of infection [(1)]. It was estimated that 200 million people are infected with schistosomiasis; in South America, Africa and Asia and 85% of them live in Sub-Saharan Africa. Approximately 280,000 die from schistosomiasis each year in Sub-Saharan Africa [(2)]. Schistosomiasis is endemic in many areas in Sudan, such as the irrigation schemes in Gezira, Rahad, Sugar cane Schemes of Khashm Elgirba, Kenana, Guneid and Assalayia [(3)]. The Blue Nile Health Project (BNHP) was established in 1979 in Gezira Scheme. The 10 year project was aimed at control of the major water and irrigation associated disease prevalent in the agricultural communities along the Blue Nile River in the Sudan, The first epidemiology survey by BNHP in 1981 revealed an a average infection rate with *S. mansoni* of 51% in 28 villages with a range from 30% to 70% BNHP 1981[(4)]. The prevalence of Schistosomiasis was 72.8% in Gezira irrigated agricultural scheme [(5)], the prevalence of *S. mansoni* infection in Gezira and White Nile region may reach up to 70 % [(6).]

MATERIALS and METHODS

Study Area and Population:

The selected area for the study was Al-Thawra Mouby village, which is one of the villages of the Gezira Scheme. It is located in the suburbs of south Wad Medani town, the capital of Gezira State. Most of the residents did not have latrines in their houses; therefore, they urinated and defecated along the bank of the main canal on the western side of the village. Fifty percent of the residents got water from the same canal. Water was kept in reservoirs to more than three or four days for the daily use. The majorly of male residents were farmers, while minority group was employed in the minor jobs in Wad Medani markets such as porters, shoe polishers and buses' conductors and the other group was employed in Wad Medani industrial area in welding and car workshops as mechanics. Some of the women were engaged in selling food and hot drinks under trees and corners or house servants in the town while some work inside the village. There are two basic schools in Al-Thawra Mouby, one of them is Al-shaikh Gasim Allah Zaiyd Basic Mixed School.

Sampling and Sample Size:

There were 438 students in the Al-shaikh Gasim Allah Zaiyd Basic Mixed School and they were all included in this study. The total number of this comprehensive sample was 438. Samples of stools were collected from each student after informed consent.

Diagnostic Techniques for *S. mansoni*:

Questionnaire as a diagnostic method:

The questionnaires were distributed for all students included in the study. The questionnaire included: personal data such as (Name, age, sex, tribe and residence), clinical symptoms such as fever, headache, abdominal pain, and previous history of schistosomiasis, water contact and children's knowledge of schistosomiasis. Responses to the questionnaire were set as scale ranging from 1 to 11 according to the level of the parameter under question. The negative response, in *S. mansoni* was considered as equal 7 while the positive responses were classified into three levels: mild 8~9, moderate 10~11 and severe >11).

Faecal Examination (Kato-Katz):

Faecal samples collected from 438 pupils were examined immediately after collection by Kato-Katz technique [7]. About fifty mg from stool samples were taken using a spatula and placed on a piece of absorbent paper. A mesh was placed on the feces and pressed with the spatula so that part of the feces material passed through the mesh. Faeces that had passed through the mesh were taken by the spatula and placed on an orifice of a perforated plate, then the feces were pressed into the orifice of the plate until it was filled (6 mm diameter hole), and a glass slide was placed under the perforated plate, so that the faecal material was uniformly spread on the microscope slide. A cover-glass was placed on the faecal material. Each sample was examined by Kato-Katz on three slides, the result was obtained by using the mean of egg count for the three slides and taken the average number as a count of eggs per gram [8].

Ultrasonography:

203 school children were selected randomly from the 438 and were examined by ultrasound for S. *mansoni* diagnosis. U/S was performed by an ultrasonographist; using convex scanner 3.5 MHz convex probe, Aloka (5100). Diagnosis of *S. mansoni* infection was evident by liver fibrosis. Liver grades classified into seven grades according to the severity; grade (A); grade (B); grade (B+); grade (C) grade (D), grade (E) and grade (F). Grade A and grade B were negative for *S. mansoni* grade B+ was mild, grade C was moderate, grade D, E and F were severe[9].

Molecular Technique:

Fifty three fresh fecal samples (50 negative for *S. mansoni* by Kato-Katz and 3 positive were taken as a control) were selected randomly for DNA extraction and further Polymerase Chain Reaction (PCR) performance and then preserved in a -70˚C fridge. DNA was extracted for *S. mansoni* eggs from the stool samples using Qiagen Kits (QIAamp DNA Stool Mini Kit). The QIAamp DNA Stool Mini Kit obtained for the rapid purification of DNA, it was generally used for specimens of 220 mg or over, and it was suitable for both fresh and frozen samples [10].

For PCR, primers were designed to amplify the 121-bp tandem repeat DNA sequence. The primers SEQ ID NO 2 SmF 5' - GATCTGAATCCGACCAACCG-3' and SEQ ID NO 3 SmB 5' - ATATTAACGCCCACGCTCTC-3' were used [11]. Two µL of DNA sample were used as template for the PCR amplification. All reactions were

carried out in Thermal cycle. The PCR master mix was performed in a total volume of 20-µL mixture containing: 20 mM Tris-HCl (pH 8.4), 50 mM KCl buffer; 1.5 mM $MgCl_2$; 0.5 µM of each primer; 200 µM dNTPs and 0.75 U from *Taq* DNA polymerase. PCR mix consisted of 3.2 µl PCR Bo, 0.8 µl $Mgcl_2$, 2µl dNTPS, 2µl primer1, 2µl primer2, 0.2µl *Taq*, 2 µl DNA, and 7.8µl sterile H_2O.

After the master mix was performed; it was placed in Gene Amp PCR machine (9600II). The PCR programme was initial denaturation at 95 ^{o} C for 5 minutes for 1 cycle, denaturation at 95 ^{o}C for 45 second, and annealing at 62 ^{o} C for 30 second and extension at 72 ^{o}C for 30 second, for 35 cycles and a final extension cycle at 72 ^{o}C for 5 minutes.

2% agarose gel was prepared and stained. Ethidium bromide was added to dissolve agarose gel and mixed. The mixture was poured into the casting tray after the comb was placed. The gel was left for 10-15 minutes to cool and polymerized. After that the comb was removed and a 1x TBE B^{o} was poured onto the tray containing the gel. 15 µL of the PCR product was mixed with a loading dye (Bromophenol blue) and loaded into wells Electrical current was applied at 120 Volt/35~40 A. The gel was visualized using gel documentation system (GDS) for the presence of 121 bp band that confirm the presence or absence of a 100 bp was also loaded in the gel that used to identification of the 121 bp band.

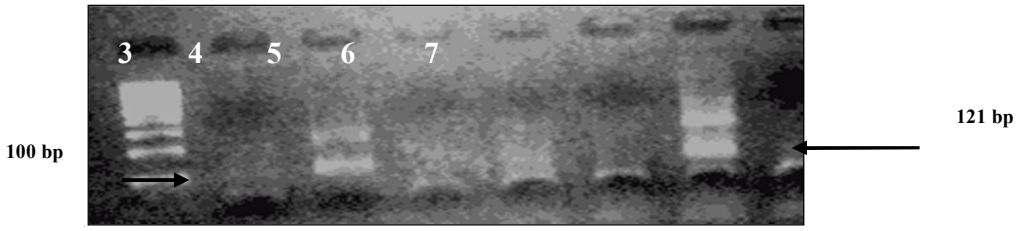

Figure 1: PCR Electrophoresis for S. mansoni Lane 1: DNA marker (100bp), Lane 2: negative control, Lane 3: positive control, Lane 4, 5, and 6: negative sample Lane 7: positive sample (negative Kato-Katz sample for S. mansoni).

Data Analysis and Statistic:

The collected data was analyzed using SPSS 16.0 (Statistical Package for Social Science) program. *p*. value was calculated using Chi- square test (χ^2) with Yates correlation. The sensitivity, specificity and positive predictive value were calculated manually by followed equations: the sensitivity = true positive / (true positive +false negative) x 100 = (—) %, Specificity = true negative / (true negative + false positive) x 100 = (—) % and positive predictive value = true positive / (true positive + false positive) x 100 = (—) %.

RESULTS

Description of the Study Subjects:

The mean-age of the school children was 11.42 ± 2.588 (minimum 6 and maximum 20 years). 192 (43.9%) were males and 246 (56.2%) were females. The study subjects were classified into three age groups; 6-10, 11-15 and >16 years old. The 11-15 years old was the most frequent age range in both males 100/192 (52.1%) and females 125/246.

Journal of Natural and Medical Sciences (JNMS)
vol. **15** (2) 2014
ISSN 1858-6805
e-ISSN 1858-6813

Table 1: overall prevalence of S. mansoni using different diagnostic techniques

Diagnostic Techniques	Males N=192	Female N=246	*P.* value
Questionnaire	47/192 (24.5%)	72/246 (29.3%)	0. 264
Kato-Katz	55/192 (28.6%)	73/246 (29.7%)	0.240
Ultrasound	54/90 (59.3%)	94/113 (43.4%)	0.023
PCR	6/25 (24%)	7/25 (28%)	0.922

Comparison between Kato-Katz and Other Techniques in Diagnosis of *S. mansoni*:

In comparison of the gold standard technique Kato-Katz with other diagnostic technique for *S. mansoni* questionnaire true positive results were 44 and false positive as 75 with true negative 235 and false negative 84 from 438 students. There was statistically significance *p*. value=0.0258 using χ^2. In comparison of PCR with the Kato-Katz, the true positive results were 3 and false positive 13 with true negative 37 and false negative 0 from 53 students. There was statistically significance *p*. value=0.0239 by Fisher's exact test. The positive predictive value (PPV) and negative predictive value (NPV) for *S. mansoni* diagnostic techniques, in questionnaire tool were 37% and 74% respectively, in diagnosis by U/S; the PPV was 30% and the NPV was 74%. Using PCR technique PPV 18% and NPV was 100%

Table 2: Comparison between Kato-Katz and other techniques

Techniques	True		False		Total	*P.*	PPV	NPV
	+ ve	- ve	+ ve	-ve				
Questionnaire	44	235	75	84	438	0.02	37	74
Ultrasound	31	75	72	26	204	0.5	30	74
PCR	3	37	13	0	53	0.02	18	100

Sensitivity and Specificity of *S. mansoni* Diagnostic Techniques:

Comparison of Kato Katz as a gold standard method with diagnostic techniques for *S. mansoni*; the sensitivity of questionnaire was 34.4% and specificity was 75.8%, the sensitivity of PCR was 100% and specificity was 74 %.

Table 3: Sensitivity and specificity for the diagnostic techniques

Diagnostic Techniques	Sensitivity (%)	Specificity (%)
Questionnaire	34.4	75.8
Ultrasound for liver	54.4	51.0
PCR	100	74

DISCUSSION

In this study *S. mansoni* prevalence was 29.2%. In a previous study in Gezira area, 50% of populations were infected with *S. mansoni* (Tayeba El Sheikh El Gorashi situ village area) ([4]. The highest incidence of *S. mansoni* infection at age range ≥16 yrs 12.7% in males and 6.8% females, the same result was found in Halfa Aljadidah females were (5.2%) at 8-18 yrs and males were (17.3%)[(12)]. The variation

in prevalence among genders may be due to different ages. Diagnosis of *S. mansoni* by questionnaire showed that there was no significant difference between males (24.5%) and females (29.3%) *P*= (0.264) while a study in Egypt showed that there is a highly significant difference (*P*<0.0001), 33.5% in males and 27.5% females [13]. Questionnaire of *S. mansoni* had a low sensitivity (34.4%) and high specificity (75.8%) with PPV 36%, in contrast that in western Côte d' lvoire had high sensitivity 88.2%, and moderate specificity 57.7% with PPV 73.2 % [14]. In China *S. japonicum* had high sensitivity (86%) and high specificity (98%) [15], the low sensitivity in diagnosis of *S. mansoni* by questionnaire may be due to invisible blood on feces as haematurea, in addition to information bias. The findings of ultrasound for *S. mansoni* diagnosis showed moderate sensitivity (54.4%) and moderate specificity (51.0%) with a positive predictive value 30% while in a study in Italy, the sensitivity was low (16%) and highly specificity (100%) with PPV 100% [16]. The dissimilarity may be due to the complications of schistosomiasis which do not appear clearly in the early childhood or that the school children responded to mass chemotherapy with a new infection. Diagnosis of *S. mansoni* by PCR was highly sensitivity (100%) and high specificity (74%) with PPV 18%, while in a study in Brazil it had high sensitivity (96.7%) and highly specificity (100%) with PPV 78.4% with Kato-Katz [17]. PCR assay might be a valuable alternative for diagnosing *S. mansoni* in negative samples (for Kato-Katz) and non endemic area.

CONCLUSIONS

This study concluded that the prevalence of *S. mansoni* infection was 29.2%. There was a significant difference between genders in diagnosis of *S. mansoni* by ultrasound while there were no significant differences in other diagnostic methods. Questionnaire is the simple method for screening of *S. mansoni*. PCR showed high sensitivity in diagnosis of *S. mansoni.*

RECOMMENDATIONS

PCR may be required as a confirmatory test in the endemic areas and as routine in non endemic area. Ultrasound can be used to identify the morbidity and complications of schistosomiasis to facilitate treatment.

REFERENCES

1. Hatz, C. F. (2005). Schistosomiasis: an underestimated problem in industrialized countries? *J Travel Med* **12**(1): 1-2.

2. Christopher J. L. Murray, Alan D. Lopez, and Colin D. Mathers, (2004). The global epidemiology of infectious diseases" World Health Organization. **4**(12):350-380.

3.Babiker, A. (1987). Transmission and Control of *Schistosoma mansoni* in the Gezira Irrigated Area of the Sudan". Ph. D. Thesis, Department of Zoology, Faculty of Science, University of Khartoum.

4.Babiker, A., Fenwich, A., Asim A., Daffallah, and Mutamad A. Amin (1985). Focality and Seasonality of *Schistosoma mansoni* transmission in The Gezira Irrigated Area, Sudan" *Journal of Trop Med Hyg*. **88**, 57-63.

5.Mohamed-Ali Q, N. E. Elwali, Abdelhameed, A. A. Mergani, A. Rahoud, S. Elagib, K. E. Saeed, O. K. Abel, L. Magzoub M. M and Dessein, A. J., (1999). Susceptibility to periportal (symmers) fibrosis in human *Schistosoma mansoni* infections: evidence that intensity and duration of infection, gender, and inherited factors are critical in disease progression. *J Infect Dis* **180** (4):1298-306.

6. Mudawi H., Ali, Y. and El Tahir, M. (2008). "Prevalence of gastric varices and portal hypertensive

gastropathy in patients with symmers periportal fibrosis. *Ann Saudi Med*. **28**(1):42-44.

7.Enk, M. J., Lima, A. C., Drummond S. C., Schall V. T., and Coelho P. M., (2008). The effect of the number of stool samples on the observed prevalence and the infection intensity with *Schistosoma mansoni* among a population in an area of low transmission. *Acta Trop* **108**(2-3): 222-8.

8.Berhe N., Medhin G., Erko B., Smith T., Gedamu S., Bereded D., Moore R., Habte E., Redda A., Gebre-Michael T., and Gundersen S.G., (2004). Variations in helminth faecal egg counts in Kato-Katz thick smears and their implications in assessing infection status with *Schistosoma mansoni*. *Acta Trop* **92**(3): 205-12.

9.WHO, World Health Organization J. Richter, C. Hatz, G. Campagne, N. R. Bergquist, J. M. Jenkins, (1996). "Ultrasound in schistosomiasis". A Practical Guide to the Standardized Use of Ultrasonography for the Assessment of Schistosomiasis-Related Morbidity. Second International Workshop.

10. Geoffrey, N. Gobert, Mitta Chia, Mary Duke, andDonald P McManus (2005). "Copro-PCR based detection of *Schistosoma* eggs using mitochondrial DNA markers". *Molecular and Cellular Probes* **19**:250-254.

11. Hamburger, J., N. He, Yu-Xin, X., Ramzy, R.M., Jourdane, J. and Ruppel, A. (1998). A polymerase chain reaction assay for detecting snails infected with bilharzia parasites (*Schistosoma mansoni*) from very early prepatency . *Am J Trop Med Hyg*. **59**(6):872-6.

12. Mahgoub, H. M., A. A. Mohamed, Magzoub, M., Gasim, G.I., Eldein, W.N., Ahmed, A.A., Adam, I. (2010). *Schistosoma mansoni* infection as a predictor of severe anaemia in schoolchildren in eastern Sudan" *J Helminthol* **84**(2):132-135.

13. El-Ayyat, A. A., H. A. Sayed, and El-Desoky, H. H. (2003). Pattern of water contact activities in relation to *S. mansoni* infection in rural area in Giza Governorate, Egypt. *J Egypt Publ Hlth Assoc* **78** (6-5) :417-432.

14. Utzinger J., N'Goran E. K., Ossey Y. A., Booth M., Traoré M., Lohourignon K. L., Allangba A. , Ahiba L. A., Tanner M., and Lengeler C. (2000). Rapid screening for *Schistosoma mansoni* in western Cote d'Ivoire using a simple school questionnaire. *Bull World Health Organ* **78**(3): 389-98.

15. Lengeler, C., Utzinger J., and Tanner M., (2002). Screening for schistosomiasis with questionnaires. *Trends Parasitol* **18**(9): 375-377.

16. Chiavaroli R. and P. Grima (2008). "Detection of early liver fibrosis in patients with intestinal schistosomiasis: sonographic and histologic findings in *Schistosoma mansoni* infection." *Infection* **36**(6): 585-589.

17. Pontes L. A., Oliveira M. C., Katz N., Dias-Neto E., and Rabello A. (2003). Comparison of a polymerase chain reaction and the Kato-Katz technique for diagnosing infection with *Schistosoma mansoni*. *Am J Trop Med Hyg* **68**(6): 652-656.