

Identification of COX1 gene in urinary Schistosomiasis among School Children at Al-Fasher Town Northern Darfur- Sudan

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Abstract

Background: Schistosomiasis is one of the most important human diseases in terms of morbidity and mortality particularly in tropical area. The disease is endemic in many developing countries that adds primarily on water bodies. The disease remains highly prevalent in Sudan, especially amongst rural communities.

Objective: The study was conducted to detect the frequency of schistosomiasis and to investigate possible genetic variations in *S. haematobium*, in AlFasher Town.

Methods: Urine samples were collected from 195 school children enrolled in this study and examined using microscopic and molecular analysis involved PCR amplifications and visualization (on agarose gel) detection for of possible genetic variants in the mitochondrial genes (COX1) .

Results : The study showed the frequency rate of *Schistosoma haematobium* in urine samples was 12/196 (6.1%). All the cases identified by microscopy got amplified, some were found to vary in their ability to express the mitochondrial genes COX1.

Conclusions: Out of 12, positive microscopically urine samples eight was positive by COX1 gene PCR (8/12(66.6%), This finding will therefore add to knowledge on genetic variations in *S. haematobium*.

Keywords: Urinary schistosomiasis, PCR, COX1gene.

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Introduction:

Urogenital schistosomiasis is caused by infection with *Schistosoma haematobium*, a dioecious blood fluke that utilizes specific freshwater snails of the genus *Bulinus* as obligates intermediate hosts (1). Adult female flukes lay terminal-spine eggs, which cross the bladder wall, leading to macro-hematuria or red urine. Through poor environmental sanitation control, eggs that reach freshwater subsequently hatch to release a miracidia that actively searches and penetrates a

freshwater snail where they undergo sexual reproduction, emerges. The cercaria is able to cross unbroken skin directly (2).

Water contact activities such as washing and bathing in water where cercariae are present enables the *Schistosoma* to gain entry into the human host. Indeed, any demographical groups, irrespective of age or gender with unsafe water contact are at risk of infection (2, 3).

Like all neglected tropical diseases, schistosomiasis is a disease of poverty.

The broader social environment in which people live and work is key to understand vulnerability to the disease (4, 5, 6). Although there are some studies on *S.haematobium* infections in some parts of the country there are no published reports in Darfur (7), fact that justifies the importance of conducting the study in Al Fasher Town. This study reveals *S. haematobium* infections and COX1 gene at Al Fasher Town. COX1 is a protein, also known as mitochondrially encoded cytochrome c oxidase I (MT-CO1) that in humans is encoded by the MT-CO1 gene (8).

The *cox1* gene is universally carried by the mitochondrial genome and has been acknowledged as a global standard DNA barcoding approach for identifying organisms at the species level (9, 10).

It is a highly conserved region in the mitochondrial genome (10). The *cox1* gene is very sufficient for rapid discrimination between closely related species and to evaluate intraspecific diversity among species (10, 11).

Material and Methods:

This is a cross sectional, descriptive community-based study conducted in the period from May 2017 to August 2021 in Al Fasher, Sudan. The study involved 195 school aged children (mean age 12+); all of them were males. A few of these children showed hematuria, abdominal pain and weakness symptoms. The study area was specified as: city center (Tombase ,Makraka ,Awlad Elreef), city peripheries and IDP (Internally displaced person) Zamzam and Nifasha camps.

The samples were collected between 10 am and 2 pm as this is the maximum eggs excretion period that was reported by Gray *et al.*, (2011). The containers were place into plastic bags before being in suitable cool boxes with a temperature of 4–6 °C for subsequent examination. The samples were further subjected to microscopic examination to identify the presence and intensity of *Schistosoma* eggs and the sociodemographic and associated risk factors were obtained via a standardized questionnaire.

10% formalin was added to the positive samples before transformed to Tropical Medicine Research Institute, National Center for Research.

Molecular techniques

For the preparation of DNA extraction, G-DEX Genomic DNA Extraction Kit was used. Each fixed urine samples were washed three times in 1XPBS buffered, then samples were centrifuged for 5 min at 2000 rpm to remove formalin. For obtaining the genetic material of *Schistosoma* eggs, 300µl cell lysis solution was added to the positive urine samples to lyse the cells wall of *Schistosoma* ova, samples were chilled to room temperature and 100µl PPT was added to the cell lysate and vortexed vigorously at high speed for 20 seconds. Afterward samples were put to the ice for 5 min in order to remove the protein contamination then centrifuged at 13,000 rpm for 5 minutes. After centrifugation 300µl of supernatant containing the DNA was transferred (leaving behind the precipitate protein pellet) into 1.5ml tube. Next 300 µl 100% isopropanol was added and mixed with the sample by inverting

gently several times then centrifuged at 13,000 rpm for 1 minute; the DNA was visible as a small pellet. Equal volume of 70% ethanol was added and centrifuged at 13,000 rpm for 1 minute (to remove isopropanol) and the supernatant was discarded carefully without discard the DNA pellet. Finally, the tubes were inverted on clean paper to dry from alcohol and 150µl DNA rehydration solution was added and incubated at 65°C for 30 min. Then DNA purity was measured. To detect *Schistosoma* DNA in urine sample, PCR was performed using the extracted DNA. The *Schistosoma* partial cox1 mitochondrial DNA (mt DNA) region was amplified using a universal forward primer ShbmF (Macrogen, Korea) (5'-TTTTTTGGTCATCCTGAGGTGTAT-3') with species-specific reverse primers, ShR (5'TGATAATCAATGACCCTGCAATAA-3') for *S. haematobium*, (12). PCR amplification was performed in total volume of 25 µL containing 5µl master mix ready to load (SOLIS BIODYNE FIREPoi, Estonia), 1 µl from each

primer and 5 µl DNA (13). PCR cycling conditions were subjected to an initial denaturing step of 95 °C for 3 min, followed by 30 cycles of 94 °C for 30 s, 58 °C for 1 min 30 s and 72 °C for 1 min 30 s, with a final extension of 7 min at 72 °C. Amplicons were visualized and sized on a 1.5 %.

Statistical Analysis:

Data obtained from this study were entered into Statistical Package for Social Sciences (SPSS) version 25 and analyzed using Chi square to determine the **association** between variables and risk factors. P. value less than 0.05 was considered significant.

Results:

Macroscopic examination

Among 14 samples with hematuria twelve had *S. haematobium* infection and two did not harbor *S. haematobium* eggs. 182 samples without hematuria which also found negative for the parasite. There was significant association Between hematuria and eggs. Table 1

Table (1) Occurrence of hematuria in urine samples

Haematuria	Total	Result of urine (Conc.Tech)	
		Positive	Negative
Positive	14	12	2
		85.7%	14.3%
Negative	182	0	182
		0.0%	100.0%
Total	196	12	184
		6.1%	93.9%

(P. value< 0.05(0.001))

Overall frequency of schistosomiasis were found in 12 (6.1%) of the urine samples and Microscopic examination of 196 urine samples that the remaining 184 (93%) samples were clear obtained from school children revealed that eggs (Table 2).

Table (2) Overall frequency. of *S. haematobium* in urine samples of school children

Negative	Positive	Total specimens examined
184 (93.8%)	12 (6.1%)	196

Frequency of urinary Schistosomiasis according to age group from 16 to 19 years and 4to 9 years 16.7 %. There is significant association between age group and the presence of *S. haematobium* as represented in Table (3)

The most common urinary schistosomiasis was found among the age group ranging from 10 to 15 years (66.7%) followed by the age group ranging

Table (3): Frequency of urinary Schistosomiasis According to Age Group

Age Group	Total	Result of urine (Conc.Tech)	
		Positive	Negative
4-9 Years	55	2	53
	28.1%	16.7%	28.8%
10-15 years	120	8	112
	61.2%	66.7%	60.9%
16-19 Years	21	2	19
	10.7%	16.7%	10.3%
Total	196	12	184
	100.0%	100.0%	100.0%

P =0.020

Identification of COX 1 Gene in samples positive for *Schistosoma* eggs examined by concentration technique it was found that only eight were positive by PCR COX1 gene. Among the twelve positive urine samples that were This result is showed in table 4.

Table (4): Detection of COX1 gene by PCR

Result of urine (Conc.tech)	PCR cox1 gene	
12	PCR result Positive	PCR result Negative
	8	4
12/196 (6.1%)	8/12 (66.7%)	4/12(33.3%)

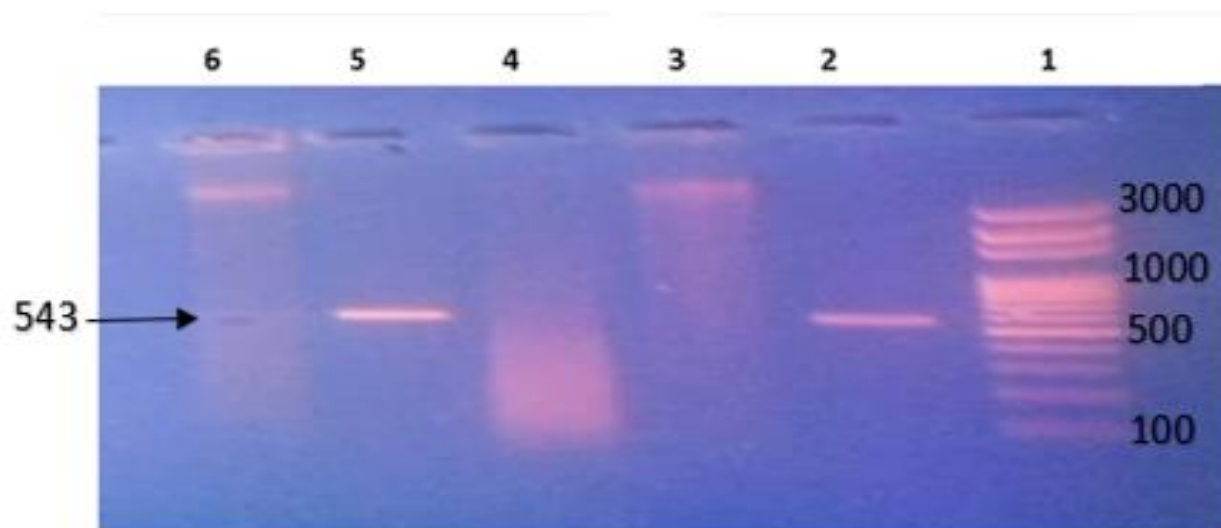


Figure 1: PCR result of *COX1* gene by gel electrophoreses. Lane 1: 100 bp DNA ladder9(1Kb+).

PCR result of *COX1* gene by gel electrophoreses. Lane 1: 100 bp DNA ladder. Lane 2 and 5: positive result (543 bp). Lane 3 and 6: non-specific bands. Lane 4: negative result. This result is showed in figure 1.

Discussion:

Human schistosomiasis is a neglected tropical disease of great importance that remains highly prevalent in Africa, especially among rural communities. (14). in this study the prevalence of *Schistosoma haematobium* was 6.1%. This finding was in agreement with study done in Egypt that was 7.8% (15), but sightless in agreement with study in Um-Asher area -Sudan (12.9%) (16), and in Southern Kordofan State 23.7% (17).

In contrast with study done in Alsafia and Abuselala South Darfur in Sudan, which found a prevalence rate of 56% (7) , and other study in White Nile River basin (Sudan) which found 45.0% (18).

In this study, macroscopic examination of hematuria showed the prevalence of 85.7% among infected patients. Statistically there was significant correlation between hematuria and the presence of *S.haematobium* eggs ($P.value= 0.001$) which it similar to in Al-Lamab Bahar Abiad Area, Khartoum State, that showed 77.8% among infected patients (19).

It was found there was insignificant association between the infection and age group which it in agreement with other study that found there was no significant relationship between the infection and the age group although other study in Al-Lamab Bahar Abiad Area, Khartoum State showed there was significant of infection with age group (19). This difference may be due to difference in sample size and diagnostic method used.

The present study showed that the children who live in Tambasae and Makraka area were the most infected (14.3% and 13.6% respectively)

compared with those who live in Nifasha and Zamzam camps (0%) this finding may be due to that the camps were designed considering the hygienic status.

Out of 12 (8/12(66.6%)), positive microscopically urine samples eight was positive by COX1 gene due to diversity to the gene other than COX1 in the current study we agreed with studies done by Angora, E., and his colleague (20) on school-aged children in different parts of Côte d'Ivoire which found 42.0% of 2164.

We disagreed with study done by Sady, H., et al (12) in Al Yamen which found 13%.

Other study by Webster, et al 2010 found that COX 1 gene is highly sensitive to discriminate *S.haematobium* and *S.bovis* which showed 100% (22).

References

1-Angelo, T., 2020. Ecology of bulinid snail intermediate hosts and transmission of *Schistosoma haematobium* among school aged children in Shinyanga district, Tanzania (Doctoral dissertation).

2-Kukula, V.A., MacPherson, E.E., Tsey, I.H., Stothard, J.R., Theobald, S. and Gyapong, M., 2019. A major hurdle in the elimination of urogenital schistosomiasis revealed: Identifying key gaps in knowledge and understanding of female genital schistosomiasis within communities and local health workers. PLoS neglected tropical diseases, 13(3), p.e0007207.

3-Stothard JR, Campbell SJ, Osei-Atweneboana MY, Durant T, Stanton MC, Biritwum N-K, et al. Towards interruption of schistosomiasis

transmission in sub-Saharan Africa: developing an appropriate environmental surveillance framework to guide and to support 'end game' interventions. Infectious diseases of poverty. 2017;6[1]:10.

4-Clark M. Female Genital Schistosomiasis (FGS): the most neglected gynaecological disease. London: The International Society for Neglected Tropical Diseases; 2017.

5- Manderson L, Aagaard-Hansen J, Allotey P, Gyapong M, Sommerfeld J. Social Research on Neglected Diseases of Poverty: Continuing and Emerging Themes. PLoSNegl Trop Dis. 2009;3[2]:e332. pmid:19238216.

6- Bruun B, Aagaard-Hansen J. The social context of schistosomiasis and its control: an introduction and annotated bibliography. 2008.

7- Kebede D., Abdeljbar E., Samir, H., Emmanuel, K., Mohamed, D. O., Alam, E M., Tanole, J., Elmonshawe, A. M., Ali, M., Gafar, A. A., Khalid, B., Abdulrahman, A., Farouq, H. High prevalence of urinary schistosomiasis in two communities in South Darfur: implication for interventions Parasites & Vectors 2011, 4:114.

8- Rostami, S., Salavati, R., Beech, R.N., Sharbatkhori, M., Babaei, Z., Saedi, S. and Harandi, M.F., 2013. Cytochrome c oxidase subunit 1 and 12S ribosomal RNA characterization of *Coenurus cerebralis* from sheep in Iran. Veterinary Parasitology, 197(1-2), pp.141-151.

9-Viricel, A. and Rosel, P.E., 2012. Evaluating the utility of cox1 for cetacean species identification. Marine Mammal Science, 28(1), pp.37-62.

- 10-** Trobajo, R., Mann, D.G., Clavero, E., Evans, K.M., Vanormelingen, P. and McGregor, R.C., 2010. The use of partial cox 1, rbc L and LSU rDNA sequences for phylogenetics and species identification within the *Nitzschia palea* species complex (Bacillariophyceae). *European Journal of Phycology*, 45(4), pp.413-425.
- 11-** Lin, X., Stur, E. and Ekrem, T., 2015. Exploring genetic divergence in a species-rich insect genus using 2790 DNA barcodes. *PloS one*, 10(9), p.e0138993.
- 12-** Sady, H., Al-Mekhlafi, H.M., Webster, B.L., Ngui, R., Atroosh, W.M., Al-Delaimy, A.K., Nasr, N.A., Chua, K.H., Lim, Y.A. and Surin, J., 2015. New insights into the genetic diversity of *Schistosoma mansoni* and *S. haematobium* in Yemen. *Parasites & vectors*, 8(1), pp.1-14.
- 13-** Dias Neto, E., Passos, L.K.F., Pereira de Souza, C., Romanha, A.J., Katz, N., Simpson, A.J.G., Steindel, M., Rollinson, D. and Pena, S.D.J., 1993. The use of RAPDs for the study of the genetic diversity of *Schistosoma mansoni* and *Trypanosoma cruzi*. In *DNA Fingerprinting: State of the science* (pp. 339-345). Birkhäuser, Basel.
- 14-** Hotez, P.J., Savioli, L., Fenwick, A. Neglected tropical diseases of the Middle East and North Africa: Review of their prevalence, distribution, and opportunities for control. *PLoS Negl Trop Dis*. 2012;6(2):e1475.
- 15-** Taha, E., Nabil G., Alan F., Rashida B., Ahmed E., Zoheir, N.M.H., Farid, A., Nabil, S., Gabr, H M.H., Mohamed, H. H., Nabil, N. H. M, Barnett, L. C., And Strickland, T.G. The Epidemiology Of Schistosomiasis In Egypt: Summary Findings In Nine Governorates. *J. Trop. Med. Hyg.*, 62(2)S, 2000. 88–99.
- 16-** Hajissa, K., Abd Elhafiz M. A. M., Hamza, A. E., Alnzer A., Elkhatieb N., Rabeea D., Safa M. A., Marwa, ., Mohamed, G., and Zeehaida, M. Prevalence of schistosomiasis and associated risk factors among school children in Um-Asher Area, Khartoum,. *BMC Res Notes* 11,(2018). 779.
- 17-** Abou-Zeid, A.H., Abkar, T.A. and Mohamed, R.O., 2013. Schistosomiasis infection among primary school students in a war zone, Southern Kordofan State, Sudan: a cross-sectional study. *BMC public health*, 13(1), pp.1-8.
- 18-** Ismail, H.A.H.A., Hong, S.T., Babiker, A.T.E.B., Hassan, R.M.A.E., Sulaiman, M.A.Z., Jeong, H.G., Kong, W.H., Lee, S.H., Cho, H.I., Nam, H.S. and Oh, C.H., 2014. Prevalence, risk factors, and clinical manifestations of schistosomiasis among school children in the White Nile River basin, Sudan. *Parasites & vectors*, 7(1), pp.1-11.
- 19-** Osman, R.M., Eisa, N.S., Nasr, A.A., Ali, O.O., Siddig, E.E. and Mohamed, N.S., 2018. Prevalence of *Schistosoma haematobium* among School Children in Al-Lamab Bahar Abiad Area, Khartoum State, Sudan 2017: A Cross Sectional Study.
- 20-** Angora, E., Vangraefschep, A., Allienne, J.F., Menan, H., Coulibaly, J., Meité, A., Raso, G., Winkler, M., Yavo, W., Touré, A. and N'goran, E., 2022. Population genetic structure of *Schistosoma haematobium* and *Schistosoma haematobium* × *Schistosoma bovis* hybrids among

school-aged children in Côte d'Ivoire. *Parasite*, 29, p.23.

21-Gray DJ, Ross AG, Li YS, McManus DP. Diagnosis and management of schistosomiasis. *Bmj*.2011; 342:d2651.

22- Webster, B.L., Rollinson, D., Stothard, J.R. and Huyse, T., 2010. Rapid diagnostic multiplex PCR (RD-PCR) to discriminate *Schistosoma haematobium* and *S.bovis*. *Journal of helminthology*, 84(1), pp.107-114.

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