



Comparative Observation of the Use of Combi 10 and Filter Paper in the Diagnosis of *Schistosoma haematobium* Infections

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Authors' contributions

This work was carried out in collaboration between all authors. Authors VUO and EUA designed the study, Authors VUO and FOI performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors VUO and EUA managed the analyses of the study. Authors VUO and FOI managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Urinary Schistosomiasis is neglected tropical disease (NTDs) caused by the trematode, *Schistosoma haematobium*. This Study was designed to comparatively observe the use of Combi 10 and filter paper in the diagnosis of *Schistosoma haematobium*, and to determine the prevalence and intensity of the Infection among Primary School Pupils in Makurdi Metropolis. A survey involving 202 pupils from four different primary schools within the Makurdi Metropolis was conducted. Urine samples were collected from pupils between the ages 5 to 19 and examined for hematuria and Ova of *Schistosoma haematobium* using Medi Test Combi 10 and Polycarbonate Filters in Urine Syringe Filtration Technique (USFT) respectively. The prevalence of *Schistosoma haematobium* based on microscopic examination of Filter papers was 25.7% while prevalence of Hematuria was 35%. Prevalence of Proteinuria was observed to be 50% Infection Intensity varied from Light to heavy. In general infection was higher among males (26.3%) than females (25.8%; $P > 0.05$) although statistically non-significant. The age specific prevalence ranged from 11.1% to 40% in 5-9 years and 15-19 years respectively ($P > 0.05$), and showed no significant difference.

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There was also a strong positive correlation between the use Medi-test Combi 10 reagent strips and Poly membrane Filtration technique. A total of 111 urine samples were positive to haematuria using combi 10 while only 52 urine samples were positive to *S. haematobium* using poly membrane filter paper, hence correlation is significant at 0.05 level. The above finding shows that *S. haematobium* is prevalent among primary school pupils and the use of Combi 10 and Filter paper are both diagnostic tools that can be used in *S. haematobium* diagnosis. They can both ascertain the prevalence of the disease will provide a guide for the treatment and eradicating of the infection. Hence, decisive control measures including administration of praziquantel to pupils are needed.

Keywords: Medi test Combi 10; polycarbonate filters; *Schistosoma haematobium*.

1. INTRODUCTION

Urinary schistosomiasis is a snail borne infection caused by a trematode, *Schistosoma haematobium*. This parasite is found in the venous plexus of the bladder, hampering the flow of blood. This leads to the burst of the veins resulting in haematuria [1]. Schistosomiasis is a Neglected Tropical Disease and estimates show that at least 206.4 million people require preventive treatment [2]. Various diagnostic techniques have been developed for the detection of Urinary Schistosomiasis. Examination of Polycarbonate filters for Ova in Urine and the use of Combi 10 reagent strips can both be used in detecting infection [3]. Hematuria and proteinuria are morbidity indicators of *S. haematobium* infection [4]. Schistosomiasis is predominant in low socio-economic communities and this is attributed to inadequate water supply in the districts, poor sanitation, and a low level of socioeconomic development. Direct and indirect diagnostic methods have been suggested to aid quick mapping surveys in endemic regions. Assortments of diagnostic techniques have been developed for detection of schistosomiasis over the past few decades, ranging from basic microscopic detection to sophisticated molecular approaches. Kosala [5] noted that the current diagnostic strategies can be grouped into the following four main categories: (i) Direct parasitological diagnosis; (ii) Immunological assays detecting stage specific antigens or antibodies; (iii) Molecular techniques detecting the DNA and RNA in serum, blood or excreta; and (iv) Use of cytokines, metabolites, and other schistosomemolecules as biomarkers [5].

Despite the availability of this wide range of diagnostic assays ranging from simple to sophisticated techniques, the detection of parasite eggs in urine (*S. haematobium*) or faeces (*S. mansoni*, *S. japonicum*) during the stage of patent infection by Microscopic

Examination remains the standard method of diagnosis of schistosomiasis [5]. Schistosomiasis can be curbed without progressing into acute stages if accurate diagnostic methods are used. Hence the use of appropriate, sensitive diagnostic tools to identify infected individuals.

2. METHODOLOGY

A cross sectional study design was conducted among school children in primary schools in Makurdi Metropolis, Benue State.

Selected Primary schools close to water bodies were visited to sensitize the students on the devastating effects of the disease and Urine Samples were collected. Published articles, journals, textbooks, paper presentations, past project and information from the internet relating to the topic were all gathered and accessed. Urine samples were collected from primary schools after which urine microscopy was carried out in search of *Schistosoma haematobium* eggs.

2.1 Ethical Considerations

A letter of introduction was obtained from the Head of Department (HOD), Department of Biological Science Benue State University. Approval was also obtained from the Benue State Ministry of Health. The school's administrators were duly informed of the objectives and benefits of the study.

2.2 Collection of Urine Specimens

Participants were strictly on voluntary bases. A total of 202 pupils volunteered thus, urine samples were collected from them, 10- 15ml of Urine specimens were collected, using clean and sterile, wide-mouthed, screw-cap plastic containers according to WHO recommendation between the hours of 10 a.m. and 2 p.m to

coincide with the period when excretion of *S. haematobium* eggs is highest .

2.3 Quality Control

Urine samples collected were transported in a dark container to the laboratory preventing them from direct light penetration in order to avoid hatching of parasite eggs before microscopy examination and the samples were processed within 24 hours after collection.

2.4 Determination of Hematuria

The freshly passed urine samples were inspected macroscopically for gross haematuria, and then screened for micro-haematuria and proteinuria using urine reagent strips (Medi-Test Combi 10). The strips were immersed in urine samples according to manufacturer's specifications. The color change was matched with standard colors by the side of the container of the reagent strips. The protocol was adopted after Poggensee et al. [6]. In addition, visible haematuria based on the appearance of bloody urine was also recorded.

2.5 Determination of *Schistosoma haematobium* Eggs

Polycarbonate membrane filters (Millipore Comp.) of 13 mm diameter and 12-14 μm pore size were used for filtration of *Schistosoma haematobium* eggs from urine.

The polycarbonate membrane filter was carefully placed on the filter support of the filter holder using a blunt-ended (untoothed) forceps [7]. The filter holder was then re-assembled and attached to the end of a Luer syringe. The plunger of the syringe was removed after which the syringe was filled with well-mixed urine sample to the 10 ml mark and the plunger was then replaced [7].

Holding the syringe over a beaker, the urine was slowly filtered through the chamber with sediments left on the polycarbonate membrane filter paper [7]. The filter holder was unscrewed and placed on specially designed racks. The filters were removed at the laboratory from filter holders using a blunt-ended forceps and transferred to a microscope slide, face upwards. Using a teat dropper, a drop of lugol's iodine was added, and then the slide was covered with a cover glass and examined

microscopically using 10x magnification with the condenser iris closed sufficiently to give good contrast. The entire filter was systematically examined for eggs of *Schistosoma haematobium*. The number of eggs in the preparation was counted and reported per 10 ml of urine. One to fifty (1-50) eggs per 10ml of urine were considered as light infection, 51-200 eggs per 10 ml of urine as moderate infection and above 200 eggs per 10 ml of urine as heavy infection [7].

2.6 Data Management and Analysis

All data were entered into an Excel spreadsheet, checked for errors and analyzed using SPSS for windows (version 15.0, SPSS Inc, Chicago, USA). Descriptive statistics was used to analyze prevalence of urinary schistosomiasis. Pearson correlation coefficient (r) was used to test the correlation between haematuria and microscopic examination outcome of urine specimens. Differences and associations were considered significant at *Precision (P)* value of <0.05 .

3. RESULTS

Ova of *S. haematobium* were identified to be large, oval, pale yellow brown in colour, and possess a characteristic terminal spine.

A total of 202 urine samples were collected from students across four primary schools within Makurdi Metropolis and examined for Ova of *Schistosoma haematobium* of which 118 were males and 84 females. The overall prevalence of urinary schistosomiasis among the students was 26.7% with 52 students testing positive to *S. haematobium* infection. Males recorded a higher prevalence of (26.3%) than females (25%).

3.1 Prevalence of *S. haematobium* Infection in Relation to Age

The age specific prevalence of *S. haematobium* among the study subjects varied from 11.1% to 40% in 5-9 years and 15-19 years age group respectively. The age groups 15-19 showed highest prevalence of 40% followed by age group 10-14 which showed a prevalence of (25.9%). The age group 5-9 showed least prevalence of (11.1%) (Table 1).

$$(\chi^2_{\text{CAL}}=8.302, \chi^2_{\text{TAB}}=5.99, P>0.05, df = 2)$$

3.2 Sex Distribution of *S. haematobium* among School Children Examined

Sex- related prevalence of *S. haematobium* infection showed a higher prevalence in males than their female counterparts as shown in Table 2. More males than females volunteered for the study. A prevalence of 26.3% was observed in males while the females showed a near prevalence of 25%. Upon chi-square analysis of the data in Table 2, there was no significant difference between the prevalence of both sexes.

$$(\chi^2_{CAL}=0.19, \chi^2_{TAB}=3.84, P>0.05 \text{ and } df = 1)$$

$$(\chi^2_{CAL}=0.19, \chi^2_{TAB}=3.84, P>0.05 \text{ and } d.f = 1)$$

3.3 Comparison between Combi 10 Reagent Strip Screening Results and Filter Paper

Out of 202 urine samples examined, 52 were positive for Ova of *S. haematobium* using Poly carbonate membrane filters, and 111 samples were positive for Haematuria. Local Government Education Authority Primary School (LGEA) showed the highest number of samples positive for both haematuria and Ova using Combi 10 and Polycarbonate membrane filters while Josephine International School showed the least as shown in Table 3.

Table 3 shows the comparison between both diagnostic methods. There was also a strong positive correlation ($r= 0.976$) between the use of Medi-test Combi 10 reagent strips and Poly membrane filtration technique. Hence correlation is significant at 0.05 levels. ($P=0.05$).

4. DISCUSSION

According to our findings, the current prevalence of Urinary Schistosomiasis in Makurdi is 52 (25.7%). This prevalence is moderate (based on definition that prevalence greater than 25.0% are moderate) [8], this is comparable with prevalence recorded in Ebonyi state which recorded a prevalence of 21.5% [9]. More so, other studies reported prevalence rates higher or even lower than our findings; Oguwuike reported a high prevalence of 45.4% in Aninri LGA in Enugu State, similarly a prevalence of 42.3% was reported in Abia State, South Eastern Nigeria [9,10]. The observed differences in the magnitude of infection may be due to geographical and socioeconomically reasons. Notwithstanding other factors that influence the prevalence of urinary schistosomiasis such as contact with water contaminated with the cercariae of the parasite, the age of study subjects may have also played a vital role in our findings. The age of study subjects ranged from 5 – 19 years.



Plate 1. Microscopic view of sample containing ova of *Schistosoma haematobium*

Table 1. Age distribution of *S. haematobium* infection among school children in Makurdi LGA

Age	Number examined	Number infected	(%Prevalence)
5-9	36	4	11.1
10-14	131	34	25.9
15-19	35	14	40
Total	202	52	25.7

Table 2. Sex distribution of *S. haematobium* among school children examined

Sex	No. examined	No infected	% Prevalence
Male	118	10	26.3
Female	84	21	25
Total	202	52	25.7

Table 3. Comparison between Combi 10 reagent strip screening results and filter paper

Location	No examined	Combi 10	Poly membrane filter
LGEA	80	52	33
Josephine	30	13	3
Daddy Memorial	53	27	8
Holy Family	39	19	8
Total	202	111	52

$r= 0.976$ $P=0.05$

The sex distribution of *S. haematobium* infection among the study subjects showed a higher prevalence in males 26.3% than their female counterparts in 25%. This is similar to a report previous report [11]. Prevalence of urinary schistosomiasis is known to be generally higher among males than in females. The gender of study participants could influence the prevalence of schistosomiasis due to variation in behavioral patterns of such persons regarding water use and contact. More males than females are predisposed to the infection due to regular and longer contact with the breeding site of the disease vectors through farming and swimming. Cardinal among other reasons for the high value is the higher tendency among males to swim, play and engage in other activities in the river and other water bodies, besides the domestic chores of washing and fetching water which exposes both sexes to infection. These studies showed that there was no significant difference in the prevalence of parasites infection between male and female school children. This difference in prevalence is however statistically not significant this could mean that within Makurdi LGA prevalence of urinary schistosomiasis or contact with contaminated water is not gender related.

The prevalence of *S. haematobium* infection in relation to age indicates that the prevalence is

highest in the age group 15-19 which have a prevalence of 40% and lowest prevalence was observed in the age group 5-9 having a prevalence of (11.1%). The difference in prevalence rate is however not significant ($P>0.05$). This could be attributed to the fact that this age group is very active and adventurous and are more likely to be involved in recreation activities such as swimming, bathing or playing in streams or waterbodies containing cercariae. This age group is more susceptible to reinfection which can occur through drinking of contaminated water, washing of clothes, swimming or bathing in streams or rivers containing the infective state of the parasite. This is similar to previous reports [12,13,14,15,16] on the prevalence of the infection in different study areas in Nigeria. The low prevalence recorded within the 5-9 age group may be due to less exposure to epidemiologic factors that predispose school children to the infection.

From this study haematuria was the predominant symptom observed among infected subjects and was observed to have a prevalence of 35%. This high prevalence rates of hematuria is similar to reports from a study conducted Minna Niger State Nigeria [15]. Proteinuria was found to be of high prevalence (50%). Both Hematuria and Proteinuria are both clinically recognized morbidity indicators of *S. haematobium* Infection,

as well as damage in the Urinary tract and kidney. *S. haematobium* may be associated with Glomeruli Pathology; when the Glomeruli are damaged, Protein and often red blood cells leak into the Urine. At present, precise origin and clinical significance of Proteinuria observed in *S. haematobium* Infection remains unknown [15]. There was a strong correlation between presence of Ova in Urine, Hematuria and Proteinuria. This conforms to earlier research [9,16].

Comparing both Diagnostic Methods the use of Filter Paper Showed more accurate results for *S. haematobium* Ova as most samples positive for hematuria using Combi 10 didn't possess Ova [5].

No *S. haematobium* ova were detected in 8.5% of the samples positive for Haematuria. This could be attributed to the fact that blood in urine may be influenced by factors other than *S. haematobium* infection such as Sick cell Anaemia, urogenital infection and damages in the liver and kidney and also acute glomerulonephritis. Also Menstruation in females may be a cause of blood in Urine.

A strong positive correlation between the use of Medi-test Combi 10 reagent strips and Poly membrane Filtration technique was observed ($P=0.05$). Thus both Techniques are reliable in the Diagnosis of *S. haematobium*. The Use of Combi 10 in detection of Hematuria is an Indirect Diagnostic method for detection of Urinary Schistosomiasis, because of its high predictive value for *Schistosoma haematobium* Infection and thus it can be used for rapid assessment. It is suitable for mass screening of Urinary Schistosomiasis; being very fast, producing immediate results and can be used to detect all infected persons who are at a risk of urinogenital disease not only Schistosomiasis.

The Use of Combi 10 is an indirect diagnostic method, in addition to the direct detection of eggs, changes in urinalysis provide important clues to the diagnosis of Urinary Schistosomiasis as it can measure semi quantitative levels of Urine blood and protein. It is cheap, simple, rapid and used for identifying people at risk of infection it provides immediate results and requires no special skill. On the other hand, of Polycarbonate Membrane Filters are Expensive, although they can be rewashed and reused. Large volume of urine is used, they provide accurate

results and can be used to measure egg output.

The standard for detection of cases of *S. haematobium* Infection is based on microscopic examination of Urine Samples for Eggs of *Schistosoma* using Polycarbonate Membrane Filter Papers, as results from these techniques are more accurate for detecting Ova. Although very expensive, these 12-14 nm filter papers can be washed and reused for multiple surveys. In line with observations of other authors [17] the use of Combi 10 (Hematuria) can be advocated for as a diagnostic tool although not alone, as presence of Haematuria may not indicate infection. It is simple and a cheap alternative for identifying a population in need of treatments. Combi 10 can be used in early and quick detection of Urinary Schistosomiasis before subjecting the samples to further screening using Polycarbonate Membrane Filter Papers.

A Comparison of the Diagnostic Methods of Urinary Schistosomiasis using Combi 10 and Polycarbonate Membrane Filter Papers showed both procedures can generate reliable and accurate data on the Prevalence of *Schistosoma haematobium* Infection.

Statistically there is a significant difference in prevalence among the four schools within the study area ($\chi^2_{CAL}=25.80$, $\chi^2_{TAB}=7.815$, $P<0.05$ and $df = 3$), with Josephine international school North Bank yielding 13.3% prevalence to the infection and LGEA primary school north Bank yielding the highest prevalence of 40%. This could be due to the fact that Josephine international school North Bank is a private school consisting mainly of wards of middle class citizens who have access to safe drinking and bathing water and is not located close to any water body. LGEA primary school North Bank on the other hand has a greater number of infected students; it was also observed to have the highest amount of students with light intensity of infection. This is due to the fact that the school is surrounded by a water body which could be contaminated with Cercariae of *Schistosoma*, most of the students being of low socioeconomic class depend on the water body for bathing, washing of utensils and drinking.

5. CONCLUSION

The results from this study reveal that Makurdi is endemic to *S. haematobium* infection having a prevalence of 25.7% amongst school children

within Makurdi Metropolis. This present study has documented a high prevalence of Urinary Schistosomiasis among primary school children. These results are an indication that Makurdi is endemic to *S. haematobium* Infection.

From the above findings it can be observed that microscopic examination of urine samples using Poly Carbonate Membrane Filters and the use of Combi 10 (Medi-test Chemical Reagent strips) for detection of Hematuria and Proteinuria are both reliable diagnostic methods for the detection of *Schistosoma haematobium* Infection and can both be used for rapid assessment and mass screening of the infection. They are both Useful in field work and epidemiological studies.

A comparison of the both diagnostic methods showed both procedures can reliably provide data on the prevalence of the disease thus showing a strong positive correlation. Therefore the use of Combi 10 (hematuria) as a diagnostic tool can be advocated in the detection of the infection.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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