Original Research Article

Comparative Diagnosis of Urinary Schistosomiasis by Microscopy and PCR in Patients in Some Selected Primary Healthcare Centres in Keffi, North Central Nigeria

ABSTRACT

Aims: This study was conducted to compare the diagnosis of urinary schistosomiasis by microscopy and PCR in patients in some selected primary healthcare centres in Keffi, North Central Nigeria.

Study Design: The study was a cross-sectional study.

Place and Duration of Study: Keffi, Nasarawa State, between March 2021 and September 2021.

Methodology: Urine samples were collected from 200 patients (29 samples from 29 patients, each from GidanZakara, Sabon Gida, Jigwada, AngwanJaba and 28 samples from 28 patients, each from Kowa, KaiboMada and Yarkadai PHCs). andPatientdemographic information about them was obtained using structured questionnaires. The ova of *S. haematobium* were detected microscopically in the samples using the standard sedimentation technique. The DNA of *S. haematobium* was extracted from the samples, amplified and detected by conventional PCR technique using type-specific primers and analysed in a 1.5% agarose gel stained with ethidium bromide. The collected data were analysed using Smith's Statistical Package (version 2.8, California, USA), with a P value of ≤ 0.05 considered statistically significant, and Cohen's Kappa (κ) was used to assess the level of agreement between the two diagnostic methods.

Results: Out of the 200 samples screened, 6(3.0%) and 15(7.5%) were positive for urinary schistosomiasis by microscopy and PCR respectively indicating fair agreement between the two diagnostic methods (κ=0.3142). There was significant association between gender and urinary schistosomiasis (*P*>0.05), as more males were infected (microscopy:5.8%, PCR:15.1%) than their female counterparts (microscopy:0.9%, PCR:1.8%). Although both age and occupation of patients were not significantly associated with the infection (*P*>0.05), however, higher rate was recorded among pupil/students (microscopy:9.1%, PCR:29.5%); students aged ≤14years (microscopy:8.0%, PCR:18.0%) were mostly affected.

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Conclusion: Our results show variations between the two diagnostic techniques, with PCR detecting higher rates of urinary schistosomiasis confirming it to be a more sensitive method for diagnosing the infection. Although the findings suggest fair agreement between the two methods, continued efforts to improve diagnostic accuracy are essential for effective disease management.

Keywords: Schistosomiasis; Microscopy; PCR; Keffi; Nigeria

1. INTRODUCTION

Urinary schistosomiasis, caused by the parasitic flatworm *Schistosoma haematobium,* is a pervasive public health issue in many parts of Sub-Saharan Africa, including Nigeria where an estimated 101.3 million people are at risk of infection and 29 million of the people being infected [1,2,3]. Characterized by its association with inadequate water and sanitation, this Neglected Tropical Disease (NTD) continues to pose a substantial burden on the health and well-being of communities, particularly in regions withinadequate water and sanitation infrastructure, where individuals are frequently exposed to freshwater harboring the larvae of *S. haematobium*[4,5]. Keffi, located in north-central Nigeria, where reliance on untreated water sources is common, is one such region grappling with the challenges posed by urinary schistosomiasis [1,6].

Comment [A3]: This sounds like S. haematobium is only common in Sub-Saharan Africa, whereas it is also common in other parts of the world including Middle East,Corsica (France)etc. Please confirm.

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Most Primary Healthcare Centres (PHCs) in Nigeria, like those found in Keffi, Nasarawa State, where schistosomiasis is prevalent, cannot perform standard laboratory diagnosis [1,7]. This is mainly due to poor funding, limited resources, inadequate ~~training~~training, and infrastructure constraints that lead to reliance on presumptive tests that are uncertain in accuracy and lack guaranteed sensitivity and specificity [8,9]. Consequently, this challenges the effectiveness of measures for preventing and controlling schistosomiasis [3].

The diagnosis of urinary schistosomiasis involves a comprehensive approach, utilizing both traditional microscopic examination and advanced molecular techniques such as polymerase chain reaction (PCR). Clinical evaluation plays a key role in identifying symptoms associated with the disease, including haematuria and dysuria[10]. However, due to the overlap of symptoms with other conditions, a definitive diagnosis relies on laboratory methods [14]. Microscopy, the conventional diagnostic tool for schistosomiasis, has been the cornerstone of disease detection for decades. However, its limitations in sensitivity, especially for low-intensity infections, and the inability to provide species-specific identification highlight the need for more advanced diagnostic methods [11].On the other hand, PCR, a molecular technique, offers a more sensitive and specific approach by detecting the DNA of the parasite [10,11]. Despite its higher accuracy, the practicality and cost-effectiveness of PCR in resource-limited settings need careful consideration.

Our study design involves the collection of urine samples from patients attending selected primary healthcare centres in Keffi, followed by a comparative analysis of diagnostic outcomes using both microscopy and PCR. By evaluating the performance of these methods against each other, we aim to highlight their respective strengths and weaknesses in terms of sensitivity. Additionally, socio-demographic and clinical data were gathered to explore potential associations between the prevalence of urinary schistosomiasis and factors such as age, gender, and occupation of the participants.

The findings of this research hold substantial promise for informing healthcare policies, guiding clinicians in their diagnostic choices, and contributing to the global effort to control and eliminate urinary schistosomiasis. By elucidating the comparative diagnostic accuracy of microscopy and PCR in a real-world setting, this study endeavours to facilitate evidence-based decision-making and foster advancements in the management of urinary schistosomiasis in Keffi, North Central Nigeria, and similar endemic regions worldwide.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in seven (7) selected PHCs (GidanZakara, Sabon Gida, Jikwada, Yarkaddai, AngwanJaba, Kawo, and KaiboMada) in Nasarawa State, Nigeria. Keffi is a town in north-central Nigeria, about 68 kilometres from Abuja, the Federal Capital Territory, and 128 kilometres from Lafia, the capital of Nasarawa State. It is 850 metres above sea level and is located between 803 degrees north latitude and 7050 degrees east longitude. The majority of the inhabitants are traders, farmers, students and those who engage in menial jobs [12].

2.2 Study Population

Male and female patients of all ages attending the seven (7) designated PHCs in Keffi for treatment who agreed to participate in the study comprise the study population. These PHCs were chosen because of their proximity to the stream, where locals go to swim, wash, and undertake other household chores, potentially exposing them to the risky water contact due to theinfected freshwater snails that host the parasite. ~~A prepared questionnaire was used to collect the participants' socio-demographic information.~~

2.3 Sample Size Determination

The sample size in this study was obtained using the formula developed by Pourhoseingholiet al.[13] for sample size calculation in medical studies at the 0.05 level of precision.

2.4 Ethical Consideration and Consent

The Keffi Local Government Council Research Ethics Committee granted permission to undertake this study (KLG/WELL/227/VOL.I/XXX). The heads of each PHC where urine samples were taken also gave their approval. Furthermore, all participants in this study fully completed and signed an informed consent form.

2.5 Data collection, Sample Collection and Processing

A prepared questionnaire was used to collect the participants' socio-demographic information.

A prepared questionnaire was used to collect the participants' socio-demographic information.Following the distribution of the questionnaire, every participant was given a 30 ml sterile wide-mouth screw-capped plastic container labelled with their identification number and informed on how to collect the urine sample aseptically in private [14]. Between 10:00 a.m. and 2:00 p.m., 200 urine samples were collected (29 from GidanZakara, Sabon Gida, Jigwada, AngwanJaba, and 28 from Kowa, KaiboMada, and Yarkadai) and were transferred in a cool box to the Federal Medical Centre, Keffi, and stored at 4^0C in the refrigerator until ready for analysis.

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2.6 Laboratory Analysis

2.6.1 Macroscopic Examination Urine Samples

All urine samples collected were examined macroscopically for colour, clarity, presence and absence of blood, odour, and specific gravity as previously described byCheesbrough[14].

2.6.2 Microscopy of Urine Samples

Direct microscopy using the urine sedimentation method was used to detect *S. haematobium* eggs in the collected samples as previously described [14]. For this purpose, 10 ml of urine was poured into a centrifuge tube and spun at 15,000 rpm for 5 minutes. The supernatant was then ~~discarded~~discarded, and the sediments were collected using a Weide pipette. Two drops of the sediments were placed on a frosted glass slide and covered with a coverslip. The slides were then examined with a light microscope at x10 and x40 objectives for the presence of *S. haematobium* eggs. Eggs were identified and distinguished from other schistosomes by the presence of a conspicuous lateral spine; they were counted, and infection intensity was recoded as number of ova/10 mL urine and categorised into light (≤50 ova/10 mL urine) and heavy (≥50 ova/10 mL urine) [15].

~~**2.6.3 Polymerase Chain Reaction (PCR)**~~

~~*S. haematobium* DNA was detected by conventional PCR technique previously described byTombo et al.[16] using specific primers (ShDra consensus primers) from the work ofLodh et al. [17]. The test was carried out at the research laboratory of the Nigeria Centre for Disease Control, Gaduwa, Abuja Nigeria.~~

2.6.3 ~~2.6.4~~Genomic DNA Extraction

The genomic DNA of *S. haematobium* was extracted and purified directly from urine samples using a commercial genomic DNA extraction kit, Qiagen QlAamp DNA Miniprep Kit (Qiagen Group, Germany), according to the manufacturer's instructions.

2.6.~~45~~ DNA Amplification and Polymerase Chain Reaction (PCR)

The extracted DNA was amplified on a programmable thermal cycler (MJ Research Inc., Water- town, USA) using a set of primers, namely ShDra1F (5′-GATCTCACCTATCAGACGAAAC-3′) and ShDra1R (5′-TCACAACGATACGACCAAC-3′) targeting the ShDra region of *S. haematobium*, according to a previously described PCR protocol [16]. The PCR reaction volume was 20 µL and consisted of 5 ng/µL of DNA template, 3 µL of nuclease-free water, 10 µL of One Taq quick load (Biolabs, Durham, North Carolina, USA) and 0.5 µM of each primer. The following PCR conditions were used: 95 ^{0}C for

5 min initial denaturation, followed by 40 cycles of 30 s at 95 ^{0}C, 30 s at 56 ^{0}C, 1 min at 72 ^{0}C and a final extension step of 7 min at 72 ^{0}C.

~~**2.6.3 Polymerase Chain Reaction (PCR)**~~

S. haematobium DNA was detected by conventional PCR technique previously described byTembo et al. [16] using specific primers (ShDra consensus primers) from the work ofLodh et al. [17]. The test was carried out at the research laboratory of the Nigeria Centre for Disease Control, Gaduwa, Abuja-Nigeria.

2.6.~~5~~Agarose Gel Electrophoresis

The PCR products were analysed in a 1.5% agarose gel stained with ethidium bromide. The size of the PCR products was estimated from the migration pattern of a 100bp to 1000bp increment plus DNA molecular marker (BIONEER Daejeon, North Korea).The expected amplicon band size of the*S. haematobium* ShDra gene was 298 bp.

2.7 Data Analysis

Data collected were analysed using Smith's Statistical Package (version 2.8, California, USA), with a P value of ≤ 0.05 considered statistically significant, and Cohen's Kappa (κ) was used to assess the level of agreement between the two diagnostic methods.

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3. RESULTS AND DISCUSSION

This current study compared the diagnosis of urinary schistosomiasis by microscopy and PCR in patients in some selected primary healthcare centres in Keffi, North-central Nigeria (Table 1). A total of 200 patients (29 each from GidanZakara, Sabon Gida, Jigwada, AngwanJaba and 28 each from Kowa, KaiboMada and Yarkadai) (Table 2), most of them female (114/200), civil servants (87/200) aged 15 to 34 years (84/200) (Table 3) were recruited and their urine samples analysed for the presence of *S. hematobium* by microscopy and PCR. The results showed that 6(3.0%) of the participants tested positive by microscopy and 194(97.0%) tested negative for urinary schistosomiasis, while 15(7.5%) samples tested positive by PCR and 185(92.5%) tested positive (Table …..). Application of Cohen's Kappa coefficient to assess the degree of agreement between the two diagnostic methods yielded a value of 0.3142, indicating fair agreement beyond chance(Table 1).

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Comment [A8]: This statement is repeating; it was included under methodology. Rather describe the results highlighted in Table 1.

Comment [A9]: Repeated again, Rather focus on describing the findings in Table 2.

Table 2 seem to be far, I think it should follow the paragraph that describe its findings.

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Table 1: Comparative Diagnosis of Urinary Schistosomiasis by Microscopy and PCR in Patients in Some Selected Primary Healthcare Centres in Keffi, North Central Nigeria.

Diagnostic method	No. examined	Outcome	
		No. positive(%)	No. negative(%)
Microscopy	200	6(3.0)	194(97.0)
PCR	200	15(7.5)	185(92.5)

Kappa coefficient(κ)= 0.3142

~~Till~~ To date, microscopy is the conventional method for diagnosing schistosomiasis [3]. However, there is a high probability of false-negative results, especially if the parasite intensity is low or if it is not performed by an expert [10,11]. This is confirmed by the results of this current study, as the microscopy method gave a lower number of positive cases (3.0%) compared to PCR (7.5%) (Figures 1, 2, 3 and 4). Interestingly, there were no false-negative results by microscopy and PCR also did not produce any false-positive results. The calculated Kappa coefficient suggests fair agreement but also indicates room for improvement in diagnostic concordance.

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These results are consistent with previous studies comparing diagnostic methods for urinary schistosomiasis. For instance,Sow et al.[18] reported varying rates of urogenital schistosomiasis, as real-time PCR detected more cases of the infection (34.6%) compared to microscopy (20.3%) in pre-school, school-aged children and women of productive age in central Senegal.In another study among children and adults in Zanzibar, qPCR also significantly increased the sensitivity of urinary schistosomiasis diagnosis from 13.3 % to 26.8 % compared to urine filtration microscopy [19]. The higher infection rates detected with the PCR technique indicate that this technique is more accurate and robust in detecting schistosomiasis even at low levels that might have gone undetected with microscopy.

Table 2: Prevalence and Distribution of *S. haematobium*infection in relation to Sampling Areaamong Patients in Some Selected Primary Healthcare Centres in Keffi, North Central Nigeriaby Microscopy and PCR

Sampling Area	**No. Examined (N=200)**	**Prevalence (%)**	
		Microscopy	**PCR**
PHC GidanZakara	29	0(0.0)	6(20.7)
PHC Sabon Gida	29	4(13.7)	5(17.2)
PHC Jigwada	29	0(0.0)	0(0.0)
PHC Angwan Jaba	29	2(6.9)	2(6.9)
PHC Kawo	28	0(0.0)	1(3.6)
PHC Kaibo Mada	28	0(0.0)	1(3.6)
PHC Yarkadai	28	0(0.0)	0(0.0)
Total	**200**	**6(3.0)**	**15(7.5)**

Interestingly, the overall prevalence of 7.5% of *S. haematobium* infection by PCR recorded in this current study is lower than the reports of most previous researchers who detected the parasite at the same study location using microscopic methods. For example, Ezhim et al.[20]andHaruna Mohammed & Buhari[1]reported 53% and 12.0% prevalence of schistosomiasis in urine of primary school pupils in Keffi, respectively. In addition,Abdullahi & Ramatu[21] also recently reported 15.3% cases of the parasitic infection among pupils of AngwanLambu primary school in Keffi. The present study has reconfirmed the endemicity of this Neglected Tropical Disease (NTD) in Keffi Nasarawa

State, which was previously associated with extreme poverty, inadequate or lack of social amenities such as portable water for drinking and other domestic activities [1,2,22].

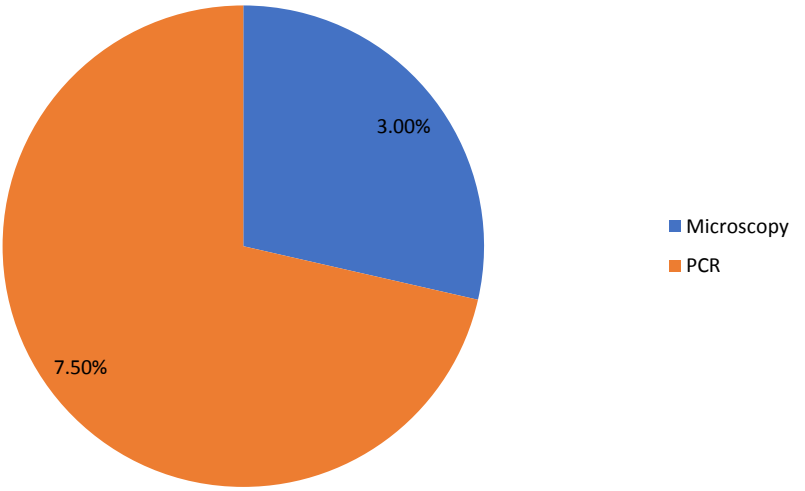

Figure 1: Comparative Diagnosis of Urinary Schistosomiasis by Microscopy and PCR in Patients in Some Selected Primary Healthcare Centres in Keffi, North Central Nigeria.

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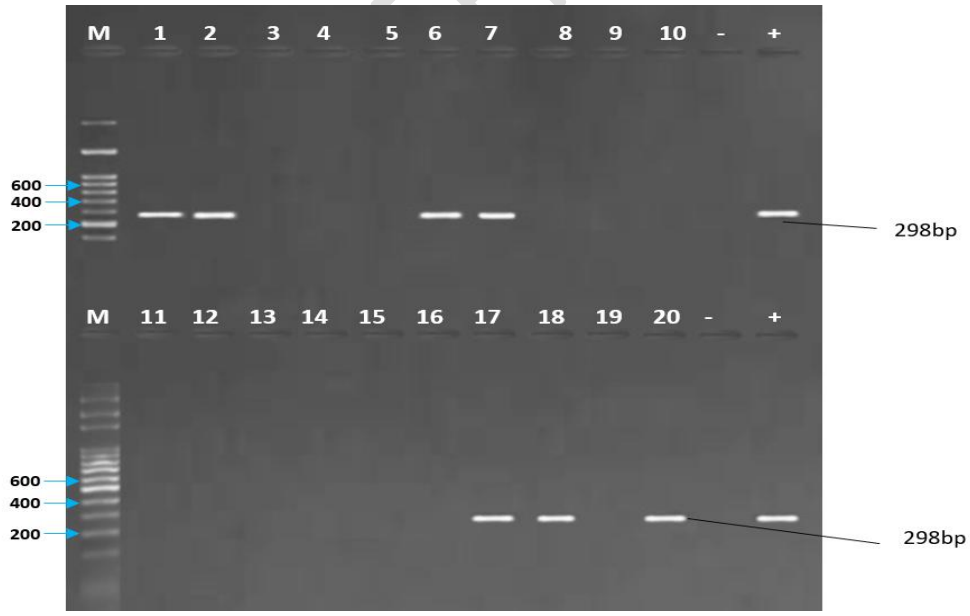

Figure 2: Agarose gel electrophoretogram of *S. haematobium*ShDra1 amplified gene. Samples 1, 2, 6, 7, 17, 18, and 20 were positive for *S. haematobium*DNA while samples 3, 4, 5, 8, 9, 10, 11, 12, 13 14, 15, 16 and 19 were negative. *M represents the molecular ladder, '–' is the negative control while '+' is the positive control.

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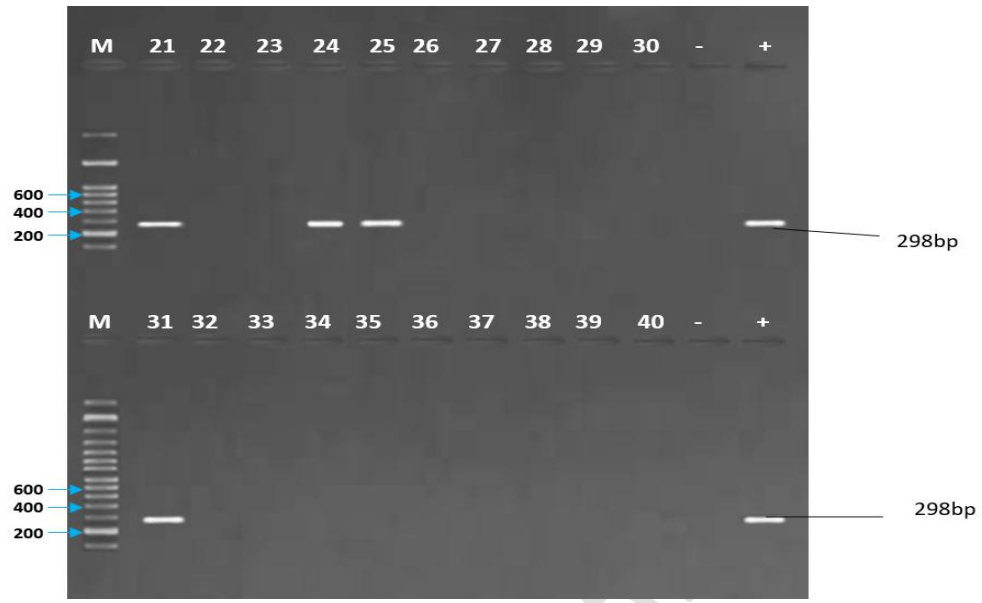

Figure 3: Agarose gel electrophoretogram of *S. haematobium*ShDra1 amplified gene. Samples 21, 24, 25 and 31 were positive for *S. haematobium*DNA while samples 22, 23, 26, 27, 28, 29, 30, 32, 33, 34, 35, 36, 37,38, 39 and 40 were negative. *M represents the molecular ladder, '−' is the negative control while '+' is the positive control.

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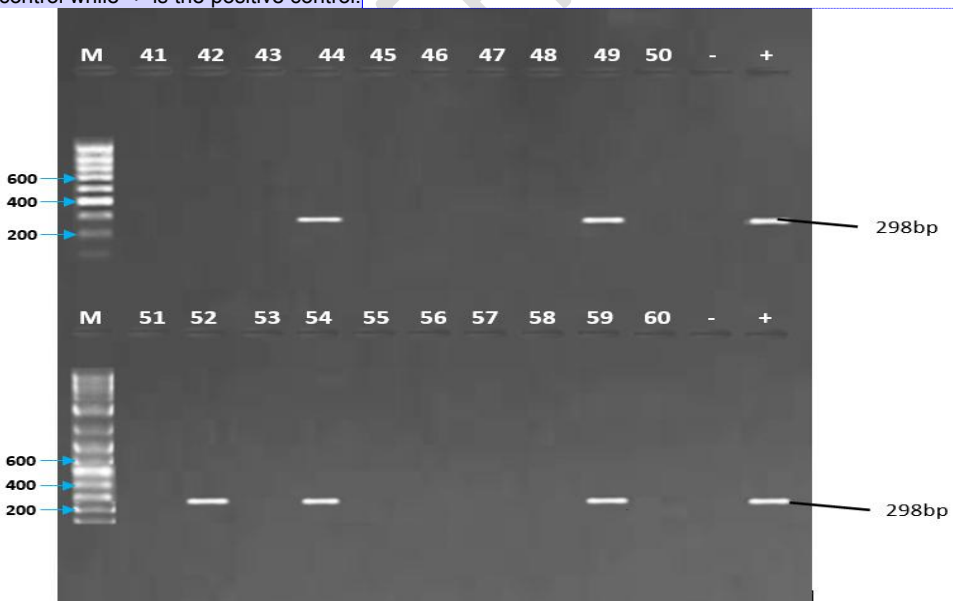

Figure 4: Agarose gel electrophoretogram of *S. haematobium*ShDra1 amplified gene. Samples 44, 49, 52, 54 and 59 were positive for *S. haematobium*DNA while samples 41, 42, 43, 45, 46, 47, 48, 50, 51, 53, 55, 56, 57, 58 and 60 were negative. *M represents the molecular ladder, '−' is the negative control while '+' is the positive control.

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The decreased rate of urinary schistosomiasis observed in this present study compared to other previous studies conducted in the same locality could be due to the integrated prevention and control measures introduced in public primary schools in Nigeria, including mass drug administration to infected individuals and health education on the predisposing factors responsible for the transmission of the parasitic infection [23]. It could also be due to the fact that most of the previous studies conducted at the study location such as those byHaruna Mohammed & Buhari[1], Ezhim et al.[20] and Abdullahi & Ramatu[21] were all carried out among school-aged children who are known to be the most susceptible groups to schistosomiasis [2,22], whereas the current study surveyed patients of all age groups.

There is no significant association between urinary schistosomiasis and the age of the participants in the present study (P> 0.05) (Table 3). However, patients aged ≤14 years were most frequently infected using both microscopy (8.0%) and PCR (18.0%). This is to be expected as school-aged children (SAC), who are usually under 14 years of age, have been shown to be the most vulnerable group for schistosomiasis [7,24, 25, 26] as they are more likely to engage in activities that may expose them to contaminated freshwater, like playing in infested water, swimming, washing, etc [4, 27]. Other previous studies also reported similar findings [1, 18, 28].

Table 3: Prevalence and Distribution of *S. haematobium*infection in relation to Socio-demographicsamong Patients in Some Selected Primary Healthcare Centres in Keffi, North Central Nigeriaby Microscopy and PCR

Socio-demographic	**No. Examined (N=200)**	**Prevalence (%)**	
		Microscopy	**PCR**
Age (Years)			
≤14	50	4(8.0)	9(18.0)
15-34	84	1(1.2)	3(3.6)
35-64	43	1(2.3)	3(6.9)
≥65	23	0(0.0)	0(0.0)
P-value		**0.1107**	**0.0850**
Gender			
Male	86	5(5.8)	13(15.1)
Female	114	1(0.9)	2(1.8)
P-value		**0.0428***	**0.0004***
Occupation			
Pupil/Student	44	4(9.1)	13(29.5)
Farmer	87	1(1.1)	2(2.3)
Civil Servant	41	0(0.0)	0(0.0)
Others	28	1(3.6)	0(0.0)
P-value		**0.0774**	**0.1000**

Statistically significant

Infection with *S. hematobium* is significantly associated with gender in this study (P < 0.05), as more male patients were infected with the parasites both microscopically (5.8%) and by PCR (15.1%) than their female counterparts (0.9% and 1.8% for microscopy and PCR, respectively) (Table 3). This could be due to the fact that most males, especially in rural areas, engage in outdoor jobs, such as farming and fishing, where they come into contact with freshwater sources, which increases their risk of infection with *S. hematobium.* Most other researchers have also made similar observations [1, 24, 26, 29].

It is interesting to note that most Nigerian farmers live in rural areas associated with extreme poverty, inadequate or lack of social amenities such as portable water for drinking and other domestic activities [2, 22], and residents of such areas are usually at risk of many infections, including schistosomiasis [1, 7, 30]. Surprisingly, however, the patient's occupation is not associated with urinary schistosomiasis in the present study (P> 0.05), and pupils/students were more frequently infected with the parasite using both microscopy (9.1%) and PCR (29.5%)than farmers (1.1% and 2.3% for microscopy and PCR, respectively) (Table 3). This observed result is most likely due to the fact that the schools in the surveyed areas might be located close to water bodies contaminated with schistosome and pupils/students may indulge in play activities involving contact with the water, making them susceptible to schistosomiasis.

4. CONCLUSION

In this current study, PCR demonstrated greater sensitivity with fair agreement compared to microscopy in detecting urinary schistosomiasis. The findings suggest that PCR may be a more reliable diagnostic tool for identifying cases of the disease in the studied population. This insight has implications for improving diagnostic accuracy and subsequent management strategies for urinary schistosomiasis in North Central Nigeria.

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ACKNOWLEDGEMENTS

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COMPETING INTERESTS

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AUTHORS' CONTRIBUTIONS

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CONSENT

All patients included in the study or their parents/guardians completed and signed an informed consent form.

ETHICAL APPROVAL

All authors hereby declare that all experiments were reviewed and approved by the appropriate ethics committeeand have been conducted in accordance with the ethical standards laid down in the 1975 Declaration of Helsinki.

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