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RESEARCH ARTICLE OPEN ACCESS

Epidemiological survey and chromatographic profiling of ligands associated with Schistosoma haematobium infection in Oke-Alafia, Ondo State, Nigeria

Ligand Profiling and Epidemiology of S. haematobium

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Abstract
Objective: Nigeria is the world's most endemic country for schistosomiasis, with the disease burden second only to malaria globally.
Objective: This study aimed to survey Schistosoma haematobium infection in Oke-Alafia, Ondo State, and identify ligands associated with urinary schistosomiasis.
Methods: A cross-sectional survey of 400 participants was conducted in the Oke-Alafia community to assess the distribution of *Schistosoma haematobium* infection and identify associated ligands. Microscopy and cultural techniques were employed on urine samples from randomly selected subjects to detect S. haematobium and exclude bacterial infections. Statistical analysis used the Chi-square test ($p < 0.05$).
Results: The prevalence of urinary schistosomiasis was 18.75%. The most abundant ligands in infected urine samples included Methyl 4-hydroxybutyl, Trimethyl Silyl, Thiazole, 2-Pyrrolidinone, 1-methyl, and Piperidine. Statistical analysis showed no significant difference in ligand abundance related to urinary schistosomiasis ($X^2 = 1.7312$; $P > 0.05$). In contrast, normal urine samples (without parasitic or bacterial infections) had five distinct ligands, including Hydrazine carbothioamide, Dihydroartemisinic acid, and Silane, with significant differences in abundance ($X^2 = 5.242$; $P < 0.05$).
Conclusion: Identified ligands may serve as potential biomarkers for rapid diagnostic methods in urinary schistosomiasis.

Keywords: Biomarkers, Chromatographic, Ligands, Microscopy, *Schistosoma haematobium*

Plain English Summary

This study looked at how common urinary schistosomiasis (a water-borne disease caused by the parasite Schistosoma haematobium) is in a Nigerian community called Oke-Alafia, and whether certain chemical markers (called ligands) in urine could help detect the infection faster.

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Out of 400 people tested, nearly 19% had the infection, mostly children under 16. Using urine analysis and a technology called GC-MS (Gas Chromatography–Mass Spectrometry), the researchers found specific chemicals in the urine of infected people that were not present in healthy individuals. These include compounds like Methyl 4-hydroxybutyl and Piperidine.
Interestingly, urine from uninfected people had a different set of chemicals. These findings suggest that these unique chemicals could serve as future tools for diagnosing the disease without needing a microscope.

Background:
Schistosomiasis, caused by trematodes of the genus Schistosoma, is globally ranked the second most devastating parasitic disease next to malaria (1, 2), affecting over 240 million people in developing countries. Nigeria is the most endemic country in the world for schistosomiasis (2, 3, 4, 5). Urinary schistosomiasis, caused by *Schistosoma haematobium*, is the most common type of schistosomiasis found in the riverine areas of Nigeria, and Bulinus species is the intermediate host (2, 6, 7, 8).
In high-prevalence settings, microscopic detection of ova in urine is a routine method for diagnosing urinary schistosomiasis (9). However, this method is time-consuming and requires expertise; therefore, rapid diagnostic techniques independent of microscopic analysis are necessary for both efficient management of the infection and a timely diagnosis, particularly in endemic regions.
Several techniques, such as Polymerase chain reaction-based, have been used to detect the parasite's DNA. However, these techniques are expensive and time-consuming (10, 11, 12). Biomarkers can serve as indicators to determine the progression of schistosomiasis (13), while the Gas chromatography-mass spectrometry method has previously been used to identify and quantify the abundance of ligands and metabolites (14).
This study aimed to conduct a survey of S*chistosoma haematobium* infection and identify ligands associated with urinary schistosomiasis in the Oke-Alafia community using Gas Chromatography Mass Spectrometry (GC-MS).

Methods
Sample Collection and Analysis
Participants were between the ages of 5 and 55 years, comprising both genders. Each participant was provided with a clean, sterile, labelled universal bottle for urine sample collection. They were oriented on how to provide up to 10ml of terminal sterile urine sample to minimise the risk of missing a scanty infection with *Schistosoma haematobium*. The sample bottles containing the urine sample were closed properly to prevent leakage or contamination and were transported to the laboratory in a cold chain as soon as possible to maintain the integrity of the samples.
Microscopy and culture techniques were conducted on the samples to identify the ova of the parasite and rule out bacterial infection. The samples were cultured on Cysteine-Lactose-Electrolyte-Deficient Agar (CLED) and MacConkey to rule out any bacilli in the family of Enterobacteriaceae associated with urinary tract infection.
The collected urine samples were examined macroscopically for the presence of haematuria. The urine samples were centrifuged gently at 3,000 revolutions per minute (rpm) for 5 minutes, after which the sediments were placed on grease-free glass slides, covered with cover slips and examined for ova of *Schistosoma haematobium* under a microscope with x10 and x40 objective lenses to determine the severity of the infection by conducting egg count.

Sample Preparation for Gas Chromatography Mass Spectrometry Analysis
Protein from the urine samples was precipitated using three per cent (3%) Sulpho-Salicylic Acid (SSA). Precipitation of protein in the urine samples was carried out by adding one millilitre of each of the urine samples to one millilitre of 3% SSA, mixing and allowing to stand for 30 minutes. Each mixture was the centrifuged at 10,000 g for 15 minutes after which the supernatant was discarded and the pellet re-suspended in 5ml of 3% SSA, followed by another centrifugation at 10,000g for 15 minutes, the supernatant was discarded and pellets were re-suspended using Trimethylsilyl derivatization agent to convert amino acids into volatile derivatives and incubated at 37℃ for 1 hour to allow for complete derivatization of the amino acids. After derivatisation, the sample was cleaned up to remove any excess derivatisation reagent or by-products that could interfere with GC-MS analysis using Solid-phase extraction.

Gas-Chromatography Mass Spectrometry (GC-MS) Analysis
Volatile derivatives from each group of samples were pooled in clean universal bottles and were injected into the GC-MS system to analyse for the

presence of ligands. The chromatogram of the Ligands was analysed using the GC-MS software. The Ligands were identified and quantified based on their retention time and relative abundance. The ligand profile in each group was noted and compared to other groups to determine the ligands associated with Urinary Schistosomiasis.

Statistical analysis

The data obtained were expressed in mean ± standard deviation (SD) and subjected to statistical analysis using SPSS Statistics for Windows, version 17.0 (SPSS Inc., Chicago, Ill., USA). Chi-square tests were used to compare the association between the prevalence of *Schistosoma haematobium* infection. P-values < 0.05 were statistically significant at a 95% confidence level.

Results

Out of 400 urine samples examined, 75 (18.75%) were tested positive for *Schistosoma haematobium* infection. The highest prevalence of urinary *schistosomiasis (*7.0%) by age was recorded amongst the age below 16 years, while the lowest prevalence rate of 0.50% was documented among people above 61 years, as shown in Figure 1 (X^2 = 0.806; P>0.05). Assessment by gender, the findings revealed a statistically insignificant higher rate of urinary *schistosomiasis* among females (9.75%) than their male counterparts, with 9.0% (X^2 = 0.9091; P>0.05)

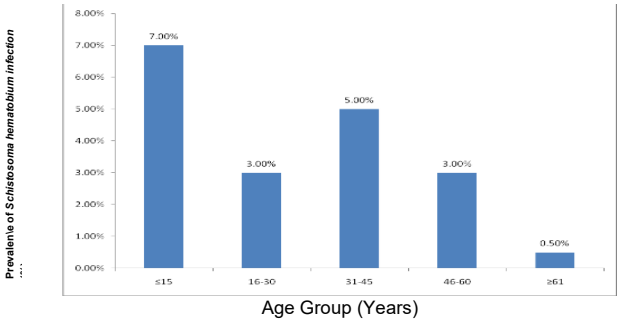

Figure 1: Prevalence of *Schistosoma haematobium* infection by age

Table 1 shows the intensity of urinary schistosomiasis to occupation. Overall, 9.5% of the infections were mild, 5.8% recorded moderate infection, while 3.8% revealed heavy density of the ova of the parasite in urine. Statistically, the findings revealed insignificant variation in the intensity of the infection by occupation (X^2 = 0.513; P>0.05)

Table 1: Intensity of Urinary Schistosomiasis by Occupation

Occupation	No. examined	No. infected	Mild	Moderate	Heavy
Students	208	42	20 (47.5)	13 (30.9)	9 (21.4)
Teachers	27	6	3 (50.0)	3 (50.0)	0 (0.0)
Farmers	104	21	11 (52.3)	4 (19.0)	6 (28.6)
Artisans	10	0	0 (0.0)	0 (0.0)	0 (0.0)
Others	51	7	4 (57.1)	3 (42.9)	0 (0.0)
Total	400	76	38 (9.5)	23 (5.8)	15 (3.8)

(X^2 = 0.513; P>0.05)

Key: Mild= 1-5 (ova / HPF); Moderate= 6-10 (ova/HPF), Heavy= >10 (ova /HPF)

The frequency of ligands in urine samples to Schistosoma haematobium and bacterial infection is as shown in Figure 2. The distribution of ligands was assessed in four groups. The first group consisted of urine samples that were positive for *Schistosoma haematobium* and the bacterial group. The second group were the samples positive for *Schistosoma haematobium* but with no bacterial growth; the third group was negative urine samples with no *Schistosoma haematobium* but with bacterial growth, while in the last group the samples were there were no Schistosoma *haematobium* and bacterial infections. Out of all the groups examined, the group with schistosomiasis but with no bacterial growth recorded the highest distribution of ligands, while the least was documented where there is co-infection of *Schistosoma haematobium* and bacteria.

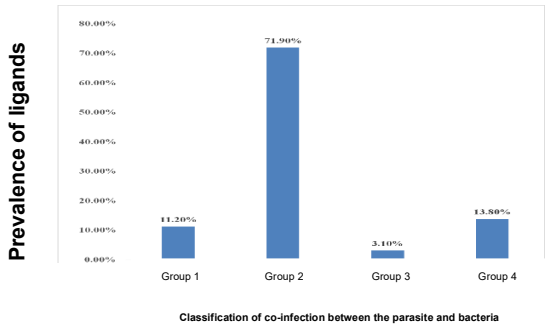

Figure 2: Frequency of ligands in urine samples concerning Schistosoma haematobium and bacterial infections

KEY: *Group 1 = Schistosoma haematobium infection with no bacterial infection; Group 2 = No Schistosoma haematobium infection and no bacterial infection; Group 3 = Schistosoma haematobium infection with bacterial infection; Group 4 = No Schistosoma haematobium infection but with bacterial infection*

Table 2 depicts the relative abundance in Urinary Schistosomiasis without bacterial co-infection.
Most copious ligands in urinary schistosomiasis were Methyl 4-hydroxybutyl, Trimethylsilyl, Thiazole, 2-Pyrrolidinone, 1-methyl and Piperidine with their relative abundance of 192000, 144000, 116000, 108000 and 104,000, respectively. Statistical analysis shows an insignificant difference in the abundance of ligands associated with only urinary Schistosomiasis *(X^2 = 1.7312; P>0.05)*.

Table 2: Ligand abundance in Urinary Schistosomiasis without bacteria co-infection

S/N	Name of ligand	Abundance in the samples	Retention time(minutes)
1	Methyl 4-hydroxybutyl	192,000	8.157
2	Trimethylsilyl	144,000	9.868
3	Thiazole	116,000	6.188
4	2-Pyrrolidinone, 1-methyl	108,000	4.054
5	Piperidine	104000	3.980

(X^2 = 1.7312; P>0.05)

Ligand abundance in a group with normal urine samples without *Schistosoma haematobium* and bacterial infections are shown in Table 3. The normal urine (without the parasitic and bacterial infection) revealed an association with another five different ligands that were not found in positive urine samples namely: Hydrazine carbothioamide, with the highest abundance, followed by Dihydroartemisinic acid, Silane, Octamethyl-2,4-Dihydroxyacetophenone and Ethenone 2-Ethylacridine recording the relative abundance of 304,000, 240,000, 104000,100000 and 72,000 respectively. Statistically, there was a significant difference in the abundance of Ligands in normal urine samples without *Schistosoma haematobium* and bacterial infections (X^2 = 5.242; P <0.05).

Table 3: Ligand abundance in normal urine samples without *Schistosoma haematobium* and bacterial infections

S/N	Name of ligand	Retention time(minutes)	Abundance in the samples
1.	Hydrazine carbothioamide	8.157	304000
2.	Dihydroartemisinic acid	9.868	240000
3.	Silane	11.390	104000
4	Octamethyl-2,4-Dihydroxy acetophenone	3.408	100,000
5	Ethenone 2-Ethylacridine	3.985	72,000

(X^2 = 5.242; P<0.05)

An abundance of ligands in urine samples infected with both the parasite and bacteria is as shown in Figure 3, while the distribution in urine samples without Schistosoma *haematobium* infection but with bacterial contamination is as depicted in Figure 4. The ligands' abundance in both groups is irregular and lopsided, without significant association with *Schistosoma haematobium* infection

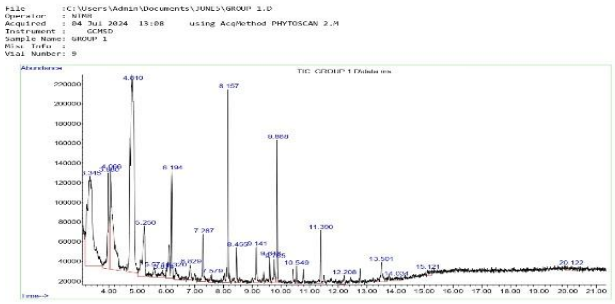

Figure 3: Chromatogram showing the abundance of ligands in urine samples with *Schistosoma Haematobium* and bacterial infections

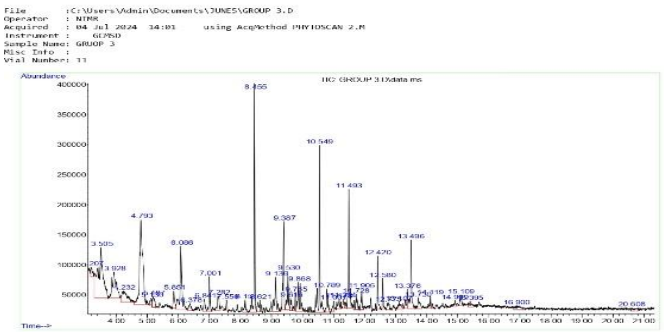

Figure 4: Chromatogram showing abundance of ligands in urine samples without *Schistosoma haematobium* infection but with bacterial growth

Discussion

The prevalence rate of *S. haematobium* (18.75%) recorded in this study is relatively low according to the standard rating of the infection in an endemic region by the WHO (15). The present result is comparable to the earlier report of Ajakaye (16) with a prevalence rate of 16.6%; however, our finding is lower than the report from Gusau in the Abarama village in the Northern part of Nigeria, where a 74.0% rate was documented (17).

Assessing this present finding on urinary schistosomiasis concerning age, a higher infection rate (7.0%) was recorded within the age group ≤15 years. This is not surprising because most of the participants within the age bracket were mostly school children, and many schools in the study area were located close to water bodies where children can swim, play and fish after school hours. The lowest rate (0.5%) was reported among older people within the age group ≥61 years. The finding is like the trend recorded in previous studies on urinary schistosomiasis in Nigeria (16, 17).

This study also depicted a slightly higher infection rate (9.75%) among female than their male counterparts (9.0%). The difference was, however, statistically insignificant ($P > 0.05$), implying that the distribution of urinary schistosomiasis is independent of gender (18, 19). This observation could be attributed to equal chances of having contact with water bodies in the community regardless of gender (16, 20).

Analysis of the rate of infection by occupation depicts artisans as being free from the parasitic infection; hence zero per cent rate was recorded among them. Overall, 9.5% of the subjects recorded mild infection, 5.8% had moderate infection, while 3.8% had heavy ones. The students recorded the highest prevalence rates in all three categories of intensity, namely: mild (47.5%), Moderate (30.9%) and heavy (21.4%). Statistical analysis shows no significant difference in the intensity of the infection according to occupation. Relatively higher prevalence rates of infection among students and farmers could be attributed to

regular contact with the water bodies in the study area. This could be attributed to the level of exposure and duration of contact with the source of infection, the water bodies. Various human activities in bodies of water could probably lead people to have intimate contact with water bodies for longer periods, as earlier reported by Akinneye et al. (1) and Ajakaye (16).

Findings from this study revealed that Methyl 4-hydroxybutyl, Trimethyl Silyl, Thiazole,2-Pyrrolidinone, 1-methyl and Piperidine were the most copious ligands associated with urinary schistosomiasis, with their relative abundance of 192000, 144000, 116000, 108000 and 104,000, respectively. while the normal urine (without ova of the parasite and bacterial infection), recorded an association with five major ligands that were not present in positive urine samples namely: Hydrazine carbothioamide, Dihydroartemisinic acid, Silane, Octamethyl-2,4-Dihydroxyacetophenone and Ethenone 2-Ethylacridine recording the relative abundance of 304000, 2000, 104000, 100000 and 72,000 respectively. The parasitic infection may probably enhance the production of Methyl 4-hydroxybutyl and the four other ligands, while Hydrazine carbothioamide and the other four were inhibited. Distribution of ligands in urine samples without *Schistosoma haematobium* infection but with bacterial contamination, as well as urine samples infected by both the parasite and bacteria, depicted no associated ligands

Ligand abundance and retention time are essential parameters in GC-MS technology, providing valuable information for compound identification and quantification (21, 22, 23). Ligand abundance is used to quantify the amount of a specific compound in a sample and to identify potential biomarkers in the sample against a particular infection (24). It also refers to the amount of a specific molecule that binds to a receptor or protein present in a sample. Retention time is the time taken for a compound to pass through the Gas Chromatography column and reach the detector (25). An abundance of ligands can influence the Retention Time since the time is often affected by the interactions between the Abundant compound and the stationary phase of the GC-MS.

Ligands play a crucial role in the interaction between the parasite and its host (26, 27). A high abundance of some major ligands associated with urinary schistosomiasis in this study could be attributed to the severity of the parasitic infection, which may lead to increased morbidity and mortality. This is because ligands can facilitate the attachment of parasites to host cells, promoting the progression of the disease (28). Studies targeting the presence of some ligands could provide a novel approach for the development of therapeutic interventions against the disease (29). The high abundance of ligands associated with urinary schistosomiasis may also have implications for diagnostic strategy, as Ligands could serve as potential biomarkers for the diagnosis of urinary schistosomiasis (12, 30).

Given the moderate prevalence rate of urinary schistosomiasis across all age groups and genders within the study area, further research on the proteins associated with the infection and how they may contribute to the rapid identification of the parasite in suspected urine samples is also advocated.

Conclusion

In conclusion, the prevalence rate of urinary schistosomiasis in the Oke-alafia community, Okeigbo, Nigeria was 18.75%, comprising 9.75% among females and 9.0% among their male counterparts. Methyl 4-hydroxybutyl, Trimethyl silyl, Thiazole, 2-Pyrrolidinone, 1-methyl and Piperidine were the most copious ligands associated with urinary schistosomiasis, with their relative abundance 192000, 144000, 116000, 108000 and 104,000, respectively. The normal urine (without ova of the parasite and bacterial infection) revealed an association with five different ligands that were not found in positive, namely: Hydrazine carbothioamide, Dihydroartemisinic acid, Silane, Octamethyl-2,4-Dihydroxyacetophenone and Ethenone 2-Ethylacridine, recording the relative abundance of 304,000, 240,000, 104000,100000 and 72,000, respectively. Further studies to identify proteins associated with urinary schistosomiasis are strongly advocated.

Declarations

Ethics approval and consent to participate

This study was a cross-sectional epidemiological survey conducted between April and October 2024 in Oke-Alafia town, Okeigbo District, Ondo State, Nigeria. Before sample collection, Ethical approval with reference number NHREC/TR/UNIMED/ONDOSt/22/06/22 was obtained from the UNIMED Ethical Review Committee. Information on socio-demography, Knowledge, Attitude and risk factors was obtained from the participants through the administration of a questionnaire.

Consent for publication
All authors gave consent for publication of the work under the Creative Commons Attribution-Non-Commercial 4.0 license.

Availability of data and materials
All essential data supporting the findings of this case are available within the article. Additional data are available upon request from the corresponding author.

Conflict of Interest
The authors declare no conflict of interest.

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Authors' contributions
ATD: Conceptualisation, project administration, writing the original draft, methodology. AAA: Literature search, writing review and editing, methodology and validation. ALI: data acquisition and analysis. BS: data acquisition and analysis.

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