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Full Length Research Paper

Endemicity of schistosomiasis in some parts of Anambra State, Nigeria

Dennis .O. Ugochukwu^{1*}, Celestine O. E. Onwuliri¹, F. O. U. Osuala¹, Ikechukwu N. S. Dozie², F. N. Opara³ and Ucheamaka Chinenye Nwenyi⁴

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Investigation on the prevalence of infections with *schistosoma* was carried out amongst inhabitants of two local government areas (LGAs), Nigeria between October 2007 and September 2008. A total of 2064 randomly selected subjects had their urine and stool samples examined for eggs of *Schistosoma* and 323 (15.7%) were excreting eggs of *S. haematobium* in their urine with geometric mean egg count (GMEC) of 10.1 eggs/10 ml of urine. No eggs of *S. mansoni* were found. Prevalence of infection varied between 12.8 and 19.8% between the LGAs, but the differences were not statistically significant ($P>0.05$). Prevalence also varied significantly between the age groups ($P<0.05$) with peak occurring among persons aged 11 to 20 years and decreased thereafter. There was a close association between haematuria and the presence of eggs of *S. haematobium* in the urine ($P<0.05$). People that tested positive for schistosomiasis were 26.4 times at greater risk ($P<0.001$) of haematuria as compared to those who tested negative. The result indicated 83.1% sensitivity and a specificity of 91.4%. Farmers (OR=2.31; $P<0.05$) were significantly at greater risk of schistosomiasis infection as compared to the artisans. The importance of these findings and their implications for control of the disease in Anambra State are discussed.

Key words: Schistosomiasis, endemicity, Anambra State.

INTRODUCTION

Schistosomiasis (also known as Bilharziasis) is the disease caused by a blood fluke (trematode) of the genus *Schistosoma*. The intermediate host is aquatic snail. Adult schistosome worms live in mammalian host. Schistosomiasis ranks second to malaria in terms of prevalence and persistence with grave public health and socio-economic importance in endemic communities (Sleigh et al., 1998; Utzinger et al., 2003; Kazibwe et al., 2006). Five species of schistosomes are recognized as important metazoan parasites of humans. They include *schistosoma mansoni*, *Schistosoma haematobium* and

schistosoma japonicum which are widespread, while *schistosoma intercalatum* and *schistosoma mekongi* have more restricted distribution (Rollinson and Simpson, 1987; WHO 2002).

S. mansoni, the causative agent of intestinal schistosomiasis, is transmitted by aquatic snail of the genus *Biomphalaria* which thrives in irrigation canals and along lake shores (Jordan, 2000). *S. haematobium* which causes urinary schistosomiasis is transmitted by aquatic snails of the genus *Bulinus*, which inhabit less permanent water bodies (Nwoke, 1989).

Urinary schistosomiasis is an infectious disease of the tropics and sub-tropics, but highly preventable. It is one of the most widely spread among the parasitic helminthic infections that affect man. It is an occupational risk encountered in rural areas of developing countries, where potable water is scarce. The disease is indicated by the presence of blood in urine and sometimes by pains on urinating or after urinating. Man contracts the disease when he comes in contact with infected water bodies while carrying out necessary daily activities such as farming, fishing, laundry, bathing and swimming (Dalton and Pole, 1978; Wright, 1988). These socio-economic activities and symptoms are not uncommon among the inhabitants of Anambra State, Nigeria, especially in the chosen Local Government Areas.

With many rivers, ponds, irrigated farming and burrow pits, Anambra State has diverse freshwater environments that offer numerous favourable habitats for aquatic snails that serve as intermediate hosts. Some symptoms and complications are associated with this disease. These include swimmer's itch, dysuria, haematuria and suprapubic pain (Nagi et al., 1999). Knowledge on the endemicity of the disease in the chosen study area is important, because of the existing physical features and lack of social amenities in the area. In Amagunze village, Enugu State, Nigeria, Ozumba et al. (1989), reported 79% prevalence of *S. haematobium*, and intensity of 49 eggs/10 ml of urine among 119 school children aged 5 to 12 years. In a survey conducted in one secondary school and three primary schools near Agulu Lake in Anambra State, Ekejindu et al. (1999) recorded prevalence rate of 11.8% with a geometric mean egg count of 9.9 eggs/10 ml of urine. In Imo State Nigeria, Nnoruka (2000) reported prevalence ranging from 14.2 to 44.9% with geometric mean egg counts of 16 to 46% eggs/10 ml of urine. Thus, this work will also serve the purpose of bridging the information gap for adequate control of the disease in the state. The objectives of this paper were to elucidate factors influencing the existence or spread of schistosomiasis and to determine the endemicity of schistosomiasis in the study areas.

MATERIALS AND METHODS

Study area

The study areas are two LGAs in Anambra State, namely, Orumba North and Orumba South. These lie between longitude 6°37'E and 7°27'E and latitude 5°40'N and 6°48'N. The area has typical semi-tropical rainforest vegetation, characterized by fresh water swamps. It has a humid climate with a temperature of about 30.6°C (87°F) and a rainfall between 152 and 203 cm annually. The major rivers in the state are Rivers Niger, Anambra, Uiasi and Ezu. But there are other smaller streams, lakes, ponds and burrow pits. The inhabitants are predominantly farmers and the area lacks pipe borne water. The inhabitants make use of water mainly from streams, rivers, and ponds for all their domestic activities and as the major sources of drinking water. The selection of the study area was

based on the long existing lack of social amenities in the areas.

Sample collection (Urine/Stool)

Informed consent was obtained from all the people before sample collection. The study was on people of ages between 0 and 60 years. This population was made up through selections from the three thousand questionnaires distributed to the schools and other relevant establishments chosen for the research. This work started with the researchers visiting the twenty-one communities in the study area for fact findings. There, some elderly people were approached for discussions on relevant issues about their communities. Questions were asked about their sources of water for drinking and other domestic activities. Information on nearness to existing streams and rivers and existence of ponds and similar stagnant waters and their public uses were sought. The occupation of the people especially the nature of farming activities was discussed. The elders willingly answered the questions. Information obtained placed sixteen communities' suspects for existence of *Schistosoma* and eliminated five for lack of *Schistosoma* friendly environments.

The choice of ten communities, five for each LGA, was made through random sampling amongst the sixteen suspect communities. Similar exercise was performed in the choice of primary and secondary schools for sample collection. Other sources of people for sampling were the LGA headquarters, health centres, people from large families and some in their work places. The questionnaires which contained necessary questions about schistosomiasis were given to the selected people from the choice schools, other chosen establishments and places. Finally, two thousand and sixty four questionnaires that were correctly answered were selected for the work. Arrangements were later made with the people for the sample collection. Following the mobilization of the communities, 2064 persons were randomly selected from the two LGAs as study participants Figure 1. A personal data form was used to obtain the following community, village, household names/code, age, sex, occupation and school. Two wide-mouthed, screw-capped, pre-numbered, sterilized plastic containers were given to each person, to collect stool and urine samples. The subjects were instructed on how to collect the stool and urine samples. Urine samples were collected between the hours of 10 am and 2 pm along with the stool samples. For urine, they were instructed to collect mid-stream urine not less than an estimated volume of 10 ml and have the last few drops of the urine passed included in the bottles. The last drops often contain the highest number of eggs (Chessbrough, 2002). Females in their monthly periods were remarked and excluded from visible haematuria counts. This was necessary to avoid false (positive) results (Savioli and Mott, 1989). Urine samples were collected between the hours of 10 am and 2 pm along with the stool samples. These were taken to the laboratory in ice-block packed coolers where they were processed and analysed.

Examination of urine for eggs

Urine examination for *S. haematobium* eggs was carried out on the 2064 samples. The standard centrifugation method as described by Cheesbrough (2002) was employed. The content of each specimen bottle was well mixed after which a sterile disposable 10 ml syringe was used to draw urine sample into centrifuge tube and this was centrifuged for 5 min at 3,000 rpm. The supernatant was decanted while the sediment was re-mixed by tapping the bottom of the tube and a little drop placed on a slide. This was covered with a cover slip and examined microscopically, using $\times 10$ and $\times 40$ objectives and eggs of *S. haematobium* identified by their possession of

Table 1. Overall prevalence of urinary schistosomiasis in the study area.

LGA	Number examined	Number (% infected)
Orumba North	1030	166 (16.1)
Orumba South	1034	157 (15.2)
Total	2064	323 (15.7)

Table 2. Prevalence of urinary schistosomiasis in Orumba North LGA.

Community	Number examined	Number (% infected)
Ajali	206	29 (14.1)
Omogho	202	40 (19.8)
Ufuma	211	37 (17.5)
Ndiowu	204	26 (12.8)
Ndikelionwu	207	34 (16.4)
Total	1030	166 (16.1)

Table 3. Prevalence of urinary Schistosomiasis in Orumba South LGA.

Community	Number examined	Number (% infected)
Isulo	199	30 (15.1)
Eziagu	198	28 (14.1)
Agbudu	213	35 (16.4)
Eri-Umuonyia	214	33 (15.4)
Oneh	210	31 (14.8)
Total	1034	157 (15.2)

terminal spine. This process was carried out on the remaining sediments. The total counts were summed up before eggs/10 ml of urine was calculated for each sample.

Examination of stool for eggs

To detect the eggs of *S. mansoni*, qualitative and quantitative analyses were done on the stool samples. For direct qualitative examination, the stool samples were first homogenized with an applicator. A light emulsion of the homogenized stool was made on the slide with normal saline using an applicator, covered gently with a cover slip. This was subsequently examined microscopically using $\times 10$ and $\times 40$ objectives, respectively. For the quantitative method, the formal-ether concentration technique as described by Cheesbrough (2002) was used.

Biomedical testing (Rapid Screening) of urine samples for haematuria

All the urine samples of the 2064 subjects were screened for blood (haematuria) except those whose blood presence was visible. Haematuria was assessed using chemical reagent strips (medi-test Combi-9). The strip is prepared to detect blood in urine. The blood detecting area contains a peroxidase compound and O-toluidine as chromogen. The principle of the test is based on the pseudo-

Table 4. Overall Age-related prevalence in the study area.

Age (years)	Number examined	Number (% infected)
0-10	243	49 (20.2)
11-20	589	127 (21.6)
21-30	549	74 (13.5)
31-40	385	39 (10.1)
41-50	223	29 (13.0)
51-60	75	5 (6.7)
Total	2064	323 (15.7)

peroxidative activity of haemoglobin and myoglobin which catalyses the oxidation of an indicator by an organic hydroxide producing a green colour. Urine with blood was recorded variously as positive (+, ++, +++) or negative when these changed from yellow to light, deep or very deep green, respectively or negative when there is no colour change. The test was performed by dipping the strip into fresh urine sample for approximately two seconds. It was removed and the tip of the strip tapped lightly on the edge of the urine container to remove the excess urine. The strip was then matched and compared with the colour chart on the label (Cheesbrough, 2002). The results of the colour changes that took place within two seconds were recorded.

RESULTS

Overall prevalence of urinary schistosomiasis in the study area

Out of the 2064 people examined for urinary schistosomiasis, 323 (15.7%) were infected. Higher prevalence was recorded in Orumba North (16.1%), than Orumba South LGA (15.2%) (Table 1). Prevalence in the study area did not differ among the two LGAs ($P > 0.05$). The prevalence of urinary schistosomiasis in five communities of Orumba North LGA is shown in Table 1. A total number of 1030 persons were examined and 166 (16.1%) showed infection due to *S. haematobium*. The highest prevalence 40 (19.8%) was observed in Omogho, while Ndiowu showed the least infection rate of 26 (12.8%). Prevalence did not differ among the communities ($P > 0.05$).

The prevalence of *S. haematobium* in Orumba South LGA is shown in Table 2. The number of people examined was 1034 and 157 (15.2%) were infected. Infection was recorded in all communities surveyed. Agbudu had the highest prevalence of 35 (16.4%), while Eziagu 28 (14.1%) had the least infection rate. Prevalence in the study area did not differ ($P > 0.05$).

Overall age-related prevalence of urinary schistosomiasis in the study area

The overall age-related prevalence of urinary

Table 5. Age related intensity in urinary Schistosomiasis in the study area.

Age (years)	No. examined	No. infected	GMEC (eggs/10 ml of urine)
0-10	243	49	11.2
11-20	589	127	14.0
21-30	549	74	10.0
31-40	385	39	8.90
41-50	223	29	7.00
51-60	75	5	2.3
Total	2064	323	10.1

Table 6. Overall prevalence of urinary schistosomiasis according to age groups and occupation of subjects.

Factor	Schistosomiasis status, N (%)		Total
	Infected	Non-infected	
Age groups (years)			
0-10	49 (15.2)	194 (11.1)	243 (11.8)
11-20	127 (39.3)	462 (26.5)	589 (28.5)
21-30	74 (22.9)	475 (27.3)	549 (26.6)
31-40	39 (12.1)	346 (19.9)	385 (18.6)
41-50	29 (9.0)	194 (11.1)	223 (10.8)
51-60	5 (1.5)	70 (4.0)	75 (3.6)
Total	323 (100.0)	1741 (100.0)	2064(100.0)
Occupation			
Farmers	117 (36.2)	485 (27.9)	602 (29.2)
Civil servants	34 (10.5)	220 (12.6)	254 (12.3)
Traders	69 (21.4)	332 (19.1)	401 (19.4)
Students/Pupils	71 (22.0)	487 (28.0)	558 (27.0)
Fishermen	23 (7.1)	117 (6.7)	140 (6.8)
Artisans	9 (2.8)	100 (5.7)	109 (5.3)
Total	323 (100.0)	1741 (100.0)	2064(100.0)

people of 0 to 10 years with 11.2 eggs/10 ml of urine. The schistosomiasis in the study area is shown in Table 4. People of ages 11 to 20 years had the highest prevalence of 127 (21.6%). Prevalence decreased gradually with increasing age. Thus persons of ages 51 prevalence. There was a significant difference in prevalence among the various age groups ($P < 0.05$).

The overall age related intensity of urinary schistosomiasis in the study area is shown in Table 5. People of ages 11 to 20 years with 14.0 eggs/10 ml of urine had the highest intensity. They closely followed by least were from people of ages 51 to 60 years with 2.3 eggs/10 ml of urine.

Of the 2064 people who participated in the study, 15.7% ($n=323$) were infected with urinary schistosomiasis,

Table 7. Logistic regression model of the predictors of urinary Schistosomiasis in the people.

Factor	Odds ratio	95% Confidence interval	P-value (2-tailed)
Age group			
0-10	3.53	1.39 - 8.94	0.005*
11-20	3.85	1.56 - 9.45	0.001*
21-30	2.18	0.87 - 5.41	0.136
31-40	1.58	0.62 - 4.01	0.518
41-50	2.09	0.80 - 5.43	0.206
51-60 †	1	-	-
Occupation‡			
Farmers	2.68	1.33 - 5.38	0.004*
Civil Servants	1.72	0.81 - 3.65	0.215
Traders	2.31	1.13 - 4.72	0.024*
Students/Pupils	1.62	0.79 - 3.30	0.258
Fishermen	2.18	0.98 - 4.85	0.059
Artisans †	1	-	-

*Significant ($P < 0.05$ or $P < 0.01$). †Reference category against which the other categories are matched. ‡Age-adjusted logistic regression analysis.

while 84.3% ($n=1741$) were uninfected. to 60 years were the least infected with 5 (6.7%) Data indicated that among those infected with schistosomiasis, majority (39.3%) were of the age group 11 to 20 years, followed by those between the age of 21 and 30 years (22.9%). The age group with the least prevalence of schistosomiasis is 51 to 60 years. With regards to the occupation of subjects, those with the highest frequency of schistosomiasis were farmers (36.2%) followed by students/pupils (22%) and traders (21.4%). The occupational group with the least prevalence of schistosomiasis is the artisans (2.8%). The frequencies of other age and occupational groups are shown in Table 7.

Logistic regression analysis indicated that subjects of age groups 0 to 10 years (odd ratio (OR)=3.53; $P < 0.01$) and 11 to 20 years (OR=3.85; $P < 0.01$) were significantly at greater risk of schistosomiasis infection compared to the age groups 51 to 60 years. However, those in age groups 21 to 30, 31 to 40 and 41 to 50 years had insignificant ($P=0.136$; $P=0.518$; $P=0.206$), greater odds (OR=2.18; OR=1.58; OR=2.09) of schistosomiasis compared to those in group 51 to 60 years. Considering the occupation of subjects, data further indicated that farmers (OR=2.68; $P < 0.01$) and traders (OR=2.31; $P < 0.05$) were significantly at greater risk of schistosomiasis infection as compared to the artisans. In contrast, civil servants, students/pupils and fishermen had insignificantly greater risk of schistosomiasis (OR=1.72, $P=0.215$; OR=1.62, $P=0.258$; and OR=2.18, $P=0.059$) as

Table 8. Overall prevalence of haematuria according to age groups amongst people who presented schistosomiasis.

Age group (Years)	Haematuria status, N (%)		Total
	Positive	Negative	
0-10	27 (16.7)	22 (13.7)	49 (15.2)
11-20	83 (51.2)	44 (27.3)	127 (39.3)
21-30	26 (16.0)	48 (29.8)	74 (22.9)
31-40	17 (10.5)	22 (13.7)	39 (12.1)
41-50	7 (4.3)	22 (13.7)	29 (9.0)
51-60	2 (1.2)	3 (1.9)	5 (1.5)
Total	162 (100.0)	161 (100.0)	323 (100)

Table 9. Overall prevalence of haematuria according to Schistosomiasis status.

Test result for schistosomiasis infection	Haematuria status, N (%)		Total
	Positive	Negative	
Positive	162 (50.2)	161 (49.8)	323 (100)
Negative	12 (0.7)	1729 (99.3)	1741 (100)
Total	174 (8.4)	1890 (91.6)	2064 (100)

compared to the artisans.

Of the 323 people who had schistosomiasis infection in this study, 50.2% (n=162) presented with haematuria, while 49.8% tested negative for haematuria. Data indicated that among those who had haematuria, majority (51.2%, n=83) were of the age group 11 to 20 years, followed by those of age 0 to 10 years (16.7%, n=27) and 21 to 30 years (16.0%, n=26). The age group with the least prevalence of haematuria is 51 to 60 years (1.2%, n=2). The frequencies of other age groups are as shown in Table 9.

Table 9 indicates that among subjects who tested positive for schistosomiasis infection, 50.2% had haematuria, while 49.8% indicated no haematuria. Amongst those who tested negative for schistosomiasis, 0.7% tested positive for haematuria, while 91.6% tested negative for haematuria.

Logistic regression analysis indicated that subjects of younger age groups (0 to 10, 11 to 20, 21 to 30, 31 to 40, and 41 to 50 years) were not at significantly greater risk of haematuria as compared to those in the age group 51 to 60 years. Data further indicated the sensitivity and specificity of the diagnostic test at each age group.

Furthermore, data indicated that subjects who tested positive for schistosomiasis were 26.4 times at greater risk ($P < 0.001$) of haematuria as compared to those who tested negative for the infection. The test result indicated

83.1% sensitivity and a specificity of 91.4%, thus indicating that the haematuria test may be a very useful diagnostic tool for the detection of infections.

Data analysis

Descriptive data was expressed as frequencies and percentages for categorical data. Logistic regression model was used to determine the predictor risk factors of urinary Schistosomiasis infection and haematuria. Statistical significance was set at $P < 0.05$. All statistics were done using Statistical Package for Social Sciences (SPSS) for windows (version 20.0).

DISCUSSION

Community prevalence

Prevalence and intensity of *S. haematobium* in this area are relatively low (15.7%) and 10.1 eggs/10 ml of urine, respectively, while *S. mansoni* is not present. According to WHO (1985), this should be considered low, but infection is wide spread in the area. The low prevalence and intensity obtained are in accord with other studies in this area such as Nale et al. (2003) in Adamawa State (11.5%), Ekejindu et al. (2002) in Anambra State (11.8%), Akogun (1986) in Malumfashi, Bauchi State (17%), Istifanus et al. (1990) in Malumfashi (17%), Okoli and Odaibo (1999) among pupils in Ibadan (17.4%), and Fajewonyomi and Afolabi (1994) among pupils in Ile-Ife, Oyo State (20.50%). Among those infected with schistosomiasis, majority (39.3%) were of the age group 11 to 20 years, followed by those between the ages of 21 and 30 years (22.9%). Analysis further indicated that people of age groups 0 to 10 years ($OR = 3.53$; $P < 0.01$) and 11 to 20 years ($OR = 3.85$; $P < 0.01$) were significantly at greater risk of schistosomiasis infection. This is further evidenced by the intensity results where people of 11 to 20 and 0 to 10 years had geometric mean egg count (GMEC) of 14.0 eggs/10 ml of urine and 11.2 eggs/10 ml of urine, respectively as compared to relatively lower egg counts in other age groups. This is because; these age groups are much more in contact with infected water bodies through swimming, laundry and other domestic and commercial activities that need streams and ponds. This is in accord with the age-related pattern of distribution of schistosomiasis in man reported by authors like Egwunyega et al. (1994), Ekejindu et al. (1999), Dunah and Bristone (2000), Daniel et al. (2001) and Nale et al. (2003). The prevalence rates among the communities in the study area ranged from 12.8% in Ndiowu in Orumba North LGA to 19.8% in Omogho for urinary schistosomiasis. The documented reports of these authors support the statement that endemicity is wide spread in Nigeria (Cowper,

Table 10. Logistic regression model indicating the influence of age and schistosomiasis infection status on haematuria,

Factor	Odds ratio	95% Confidence interval	P-value (2-tailed)	Sensitivity	Specificity
Age group (years)					
0-10	1.81	0.33 - 10.04	0.653	0.931	0.120
11-20	2.83	0.54 - 14.68	0.347	0.976	0.064
21-30	0.81	0.15 - 4.32	1.00	0.929	0.059
31-40	1.16	0.20 - 6.46	1.00	0.895	0.120
41-50	0.48	0.07 - 2.88	0.591	0.778	0.120
51-60 †	1	-	-	-	-
Schistosomiasis status					
Positive	26.46	18.87 - 37.43	0.000	0.831	0.914
Negative †	1	-	-	-	-

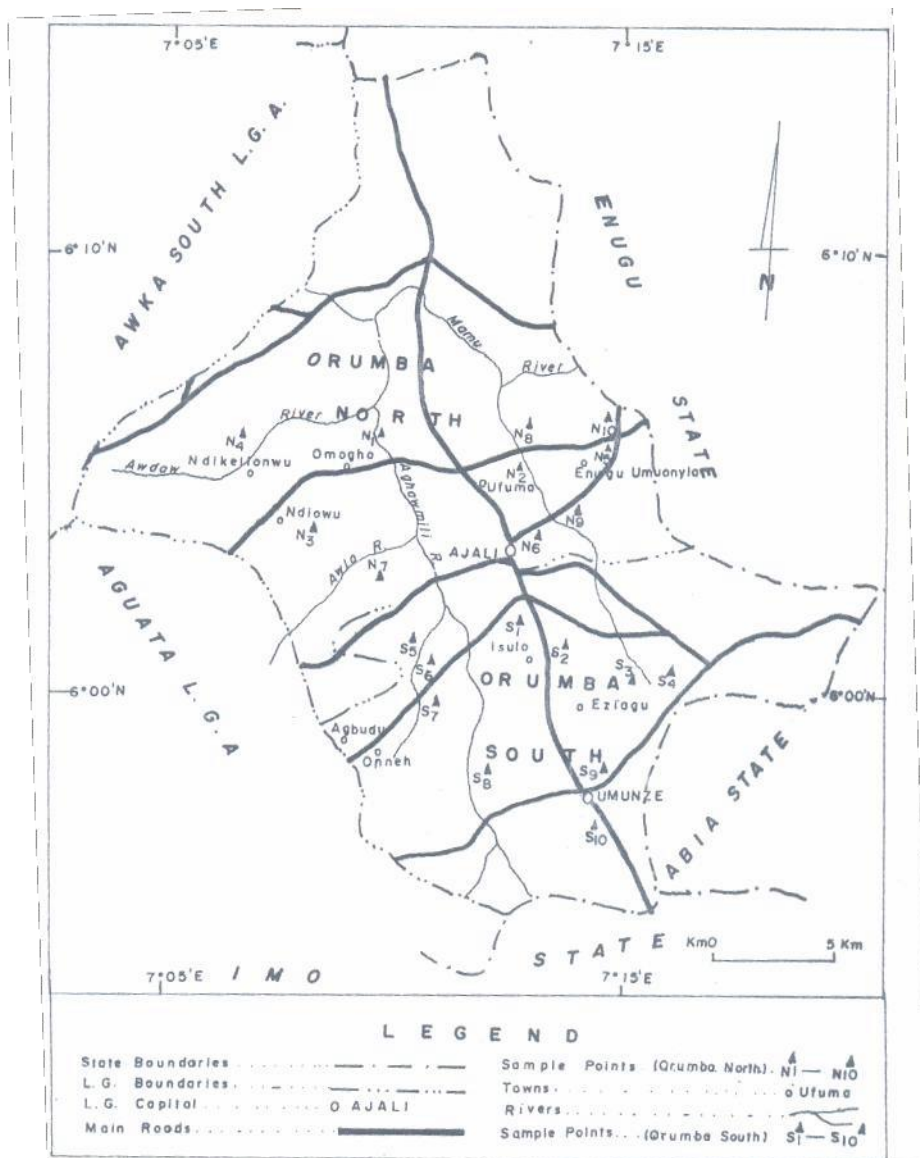


Figure 1. Map of Orumba North and South LGAs showing sampling points.

1973; Ejezie et al., 1991; Fajewoyomi and Afolabi, 1994; Ekejindu et al., 1999; Anosike et al., 1992). There was a close association between haematuria and the presence of *S. haematobium* ($P < 0.05$). Data indicated that people who tested positive for schistosomiasis were 26.4 times at greater risk ($P < 0.001$) of haematuria as compared to those who tested negative. The result further indicated 83.1% sensitivity and a specificity of 91.4% thus indicating that the haematuria test may be a very useful diagnostic tool for the detection of *Schistosoma* infections. This agrees with the reports of Akogun and Obadiah (1996), Anosike et al. (2001a) and Vender Werf et al. (2003) that had such association.

With regards to occupation of the people, those with the highest frequency of schistosomiasis, were farmers (36.2%). Data further indicated that farmers ($OR = 2.68$; $P < 0.01$) and traders ($OR = 2.31$; $P < 0.05$) were significantly at greater risk of infection. This is because farmers are unavoidably in contact with infected water due to the nature of their duty, and majority of the people in the area are farmers. Most of the traders also combine farming with trading. Considering occupation of the people, those in age groups 21 to 30, 31 to 40, and 41 to 50 years had insignificant ($P = 0.136$; $P = 0.518$; $P = 0.206$) greater odds ($OR = 2.18$; $OR = 1.58$; $OR = 2.09$) of schistosomiasis as compared to those in group 51 to 60 years. This is because these are the age groups that engage in farming business.

Conclusion

It is evident from the results that the study area is endemic for urinary schistosomiasis. This result is similar to what previous authors obtained in some other areas of the state. There is need therefore for urgent health programs aimed at controlling the infection in the state. The state government should see that pipe-born water is provided for every community in the state. Better systems of waste collection and disposal should be put in place to help make the environment more hygienic. Drugs should also be provided for those suffering from the disease.

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Full Length Research Paper

Evaluation of SD BIOLINE rapid antibody test for diagnosis of *Helicobacter pylori* infection

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Helicobacter pylori infection is most prevalent and known to cause chronic gastritis and peptic ulcer disease in Ethiopia. To date, simple and rapid point-of-care tests are commercially available; however, information is limited regarding their diagnostic significance. This cross-sectional study was conducted to evaluate the diagnostic performance of SD BIOLINE *H. pylori* rapid antibody test. A consecutive 203 enzyme linked immunosorbent assay (ELISA) confirmed sera (148 *H. pylori* positive and 55 negative) from dyspeptic patients were tested using SD BIOLINE *H. pylori* kit (Standard Diagnostic Inc, Korea) at Hawassa Teaching and Referral Hospital, southern Ethiopia from October, 2012 to January, 2013. Individuals under 15 years old, who were on anti- *H. pylori* treatment during the month prior to the study, those with discordant ELISA results, and refused to participate, were excluded. The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of the SD BIOLINE *H. pylori* test kit were 95.3, 94.5, 97.9, 88.1 and 95.1%, respectively. Therefore, this point-of-care test could be used as alternative to ELISA testing and best fit our context.

Key words: *Helicobacter pylori*, point-of-care, enzyme linked immunosorbent assay (ELISA), SD BIOLINE.

INTRODUCTION

Helicobacter pylorus is a small, spiral, gram-negative bacillus that appears to inhabit the mucous layer overlying the gastric epithelial cells in humans. It was formerly known as *Campylobacter pyloridis* then *Campylobacter pylori* (Engstrand and Lindberg, 2013). An estimated 50% of the world's population is infected; yet, its principal mode of transmission remains largely unknown. It might be due to poverty related factors that the prevalence of *H. pylori* infection is markedly higher in developing countries than in developed nations (Khalifa et al., 2010). In Ethiopia (Addis Ababa), an investigation using different diagnostic methods showed *H. pylori* prevalence varying between 69 and 91% among adult dyspeptic patients (Asrat et al., 2004). Similar high

prevalence of *H. pylori* infection (70%) was also shown in dyspeptic patients in northwest Ethiopia (Bahir-Dar) (Tadege et al., 2005). The bacterium is known as the most common cause of chronic gastritis and peptic ulcer disease. Also, infection is associated with gastric adenocarcinoma, which is the deadliest cancer (McCull and El-Omar, 2002). It has been shown that eradicating *H. pylori* infection reduces ulcer relapse (Arkkila et al., 2005) and the risk of gastric cancer development among patients with or without precancerous lesion (Wong et al., 2004, 2012).

H. pylori can be diagnosed using invasive and non-invasive methods; but a combination of at least two methods has been recommended to improve accuracy of

its detection (Ramis et al., 2012). The invasive methods such as culture, histology and rapid urease testing of endoscopic biopsy from gastric mucosa are commonly used. But these methods are tedious and time consuming and require sampling procedure that cause discomfort to patients (Cirak et al., 2007). The non-invasive methods include ^{13}C and ^{14}C urea breath tests (UBTs) and serology. UBTs are specific for active infection; however, they are more expensive or have safety concern, limiting their diagnostic suitability (Mansour-Ghanaei et al., 2011; Kato et al., 2002). In contrast, serological tests are inexpensive and widely available even though enzyme linked immunosorbent assay (ELISA) based tests require costly laboratory equipment, trained personnel and electric supply to establish diagnosis in resource-constrained settings (Leal et al., 2007).

To date, rapid diagnostic tests (RDTs) detecting antibodies against *H. pylori* infection are commercially available and widely applied in clinical practice in our settings. RDTs are simple to perform, has short test time and do not require laboratory support. However, studies reported that RDTs show varying level of diagnostic performance, which is influenced by *H. pylori* prevalence, genetic diversity and study population (Chen et al., 1997; Elitsur et al., 1997; Pelerito et al., 2006). Thus, it highlights the need to assess the diagnostic usefulness of RDTs in our context where *H. pylori* is prevalent; but little is known regarding RDT performance characteristics. This study was conducted to evaluate the diagnostic performance of SD BIOLINE *H. pylori* rapid antibody test so that its diagnostic value to serve as alternative to ELISA would be elucidated.

MATERIALS AND METHODS

Study subjects

A cross-sectional study was conducted in consecutive dyspeptic patients who provide blood for *H. pylori* serological testing at Hawassa Teaching and Referral Hospital, southern Ethiopia from October, 2012 to January, 2013. Two hundred and three ELISA confirmed sera (148 *H. pylori* positive and 55 negative) were used. There were 11 patients that were excluded: 3 were on anti-*H. pylori* treatment during the month prior to the study; 1 refused to participate; 4 had discordant ELISA results; 3 were children under 15 years old. The study obtained ethical clearance from the Institutional Review Board of College of Medicine and Health Sciences, Hawassa University.

Serology

Venous blood was collected from the study participants and sera were stored at -20°C until analyzed. All sera were tested for anti-*H. pylori* immunoglobulin G (IgG) antibody using two different ELISA kits: Pyloriset EIA-G III (Orion Diagnostica, Germany) and *H. pylori* IgG ELISA (IBL International, Hamburg, Germany). According to the manufacturers' claim, the respective sensitivity and specificity were 100 and 94.3% for Pyloriset EIA-G III and > 95 for *H. pylori* IgG ELISA. In this study, *H. pylori* infection status was determined on

the bases of the same positive or negative results obtained by both ELISA tests.

ELISAs

Pyloriset EIA-G III testing

A 100 μl of each calibrator sera (labeled 1 to 4) and 100 μl of each 1 to 201 diluted patient sera were pipetted to microtiter wells coated with the specific *H. pylori* antigen. Plates were incubated on a plate shaker (1000 rpm) at 25°C for 30 min. After washing 3 times, 100 μl of enzyme conjugate was added into the wells and incubated again at 25°C for 30 min. Following washing step, 100 μl of substrate solution was added and plates were incubated at 25°C for 10 min. The reaction was stopped by adding 100 μl of 0.5 M sulfuric acid and the optical density (OD) of each well was read at 450 nm within 10 min after stopping. As specified by the manufacturer, samples were defined as positive when their OD values are equal to or higher than that of the calibrator serum 2 OD.

H. pylori IgG ELISA testing

A 100 μl of each standard (labeled A to D) and 100 μl of each 1 to 101 diluted patient sera were added into the respective microtiter wells. Plates were covered with adhesive foil and incubated at 25°C for 60 min. After washing 3 times with washing buffer, 100 μl of enzyme conjugate was added into each well and incubated at 25°C for 30 min. Following similar washing steps, 100 μl of TMB substrate solution was added into each well and incubated at 25°C for 20 min. The reaction was stopped by adding 0.5 M sulfuric acid, and the OD of each well was read at 450/630 nm within 60 minutes after stopping. As per the instruction of the manufacturer, the OD of each sample was divided by the cut-off standard (standard B) value to obtain a cut-off index (COI). A respective COI value of < 0.8 , 0.8 to 1.2 and > 1.2 were interpreted as negative, equivocal and positive.

Rapid diagnostic test

Those ELISA characterized sera were tested using a commercial rapid diagnostic kit, SD BIOLINE *H. pylori* kit (Standard Diagnostic Inc, Korea). A single investigator blinded to the results of ELISA read all the rapid test results. Tests were carried out according to instruction of the manufacturer. The SD BIOLINE *H. pylori* antibody test is a simple one step immune-chromatographic assay for qualitative detection of antibodies of all isotopes to *H. pylori*. The test procedure is to add 10 μl of serum, plasma or whole blood to a sample well, and then adding 3 drops of assay diluent to the same well. The result was read at 10 min. Positive test result is indicated when control (C) and test line (T) are visible, and negative result is when only control line (C) is visible. The test is invalid when the control line is invisible. Data entry and analysis was performed using STATA Version-10. Results are summarized using mean and proportion as appropriate. Parameters including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy were calculated considering concordant ELISA results as true results.

RESULTS

Sera from 203 patients (102 male, 101 female) were characterized for the *H. pylori* status by ELISA testing. They

Table 1. Serology results of SD BIOLINE *H. pylori* antibody test kit, southern Ethiopia, 2012-2013.

RDT	ELISA Testing		Total
	Positive	Negative	
SD BIOLINE <i>H. pylori</i>			
Positive	141	3	144
Negative	7	52	59
Total	148	55	203

RDT, rapid diagnostic test; ELISA, enzyme-linked immunosorbent assay

Table 2. The diagnostic performance characteristics of SD BIOLINE *H. pylori* antibody test kit, southern Ethiopia, 2012-2013.

RDT	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
SD BIOLINE <i>H. pylori</i> kit	95.3 (90.5-98.1)	94.5 (84.9-98.9)	97.9 (94-99.6)	88.1 (77.1-95.1)

RDT, rapid diagnostic test; PPV, positive predictive value; NPV, negative predictive value.

were aged between 15 to 78 years, with a mean age of 36.9 years and standard deviation 14.8 years. One hundred and forty eight (72.9%) sera were positive for *H. pylori* IgG antibody and 55 (27.1%) were negative. There was an agreement between the SD BIOLINE *H. pylori* and ELISA results in 193 of 203 sera (overall accuracy 95.1%). As presented in Table 1, 141 true-positive, 52 true-negative, 3 false-positive and 7 false-negative results was obtained by SD BIOLINE *H. pylori* test kit. Out of 144 positive results, 8 were faint positive (Figure 1). The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of SD BIOLINE *H. pylori* rapid test were 95.3, 94.5, 97.9 and 88.1%, respectively (Table 2).

DISCUSSION

In our context where lack of resources and expertise limit regular evaluations of diagnostic methods, many new tests are marketed and used directly without prior evaluation for their appropriateness. Rapid serological kits for *H. pylori* are among the most widely used tests as these products are easily applicable and other diagnostic methods are limited. However, the need to determine their diagnostic value through evaluation should be emphasized for the improved management and control of *H. pylori* infection. In attempt to find a reliable point-of-care test, we evaluated the diagnostic performance of SD BIOLINE *H. pylori* rapid antibody test. The sensitivity, specificity, PPV and NPV of the test kit were 95.3, 94.5, 97.9 and 88.1%, respectively. The kit's manufacturer claimed similar performance characteristics: sensitivity

(95.9%), specificity (89.6%), PPV (93.4%), and NPV (93.5%). Also, the overall accuracy (95.1%) we reported was in accordance with that of the manufacturer (93.4%).

The various diagnostic products, dissimilar methodologies and diverse studied population may limit direct comparison between findings coming out of validation studies. In agreement with our findings, a previous evaluation of the Assure *H. pylori* rapid serological test (Genelabs Diagnostics, Singapore) reported a respective sensitivity and specificity of 90.1 and 80.9% in Bangladeshi adult patients (Pelerito et al., 2006). Moreover, evaluation of the Assure *H. pylori* in Portuguese pediatric population showed compatible specificity (95%) to that of the SD BIOLINE *H. pylori* kit, although its sensitivity (75.7%) was inferior (Rahman et al., 2008). However, unlike our evaluation, those studies included methods such as culture, rapid urease test, and histology to confirm patients' *H. pylori* status.

The NPV of SD BIOLINE *H. pylori* kit suggests that negative results could be used to rule out *H. pylori* infection. Also, the observed high PPV of the test kit indicates the reliability of positive results to detect *H. pylori* exposure, truly. Nonetheless, the faint positive results may increase inter-observer variation and the risk of reporting false-negative result. However, since the predictive values of a test depend on the prevalence of *H. pylori* infection (Banoo et al., 2010), the test kit may demonstrate different performance elsewhere if the prevalence is lower.

Serology is a non-invasive method detecting antibody against *H. pylori* infection and it is the only test unaffected by changes in the stomach that could lead to a low bacterial load and to false-negative results of the other



Figure 1. Photograph of SD BIOLINE *H. pylori* rapid antibody test results. Left: faint positive; Middle: Positive; Right: Negative (61, 94, and 96: Patients' identification numbers).

tests (Malfertheiner et al., 2012). However, serology is unable to distinguish between active infection and past exposure due to the fact that antibodies against *H. pylori* remain elevated for longer period after the disappearance of the infection (Ekstrom et al., 2001). In Ethiopian setting with a high prevalence of *H. pylori* infection (Asrat et al., 2004; Tadege et al., 2005), ELISA was shown to be highly accurate in diagnosing dyspeptic patients (Asrat et al., 2007). However, ELISA-based *H. pylori* serological test takes longer time, requires trained staff and a laboratory set-up; thus, it has application difficulties in resource-limited developing countries (Leal et al., 2008). The SD BIOLINE *H. pylori* rapid antibody test addresses challenges related to ELISA as it is simple to test, has short test time (less than 30 min), does not require laboratory equipment or electricity supply, and use whole blood, plasma or serum as diagnostic sample.

However, the results of this study should be interpreted in light of its methodological limitation as the tests employed for *H. pylori* diagnosis did not include methods of choice such as culture, UBTs and histology against those comparisons of the serological tests would have been imperative.

Conclusion

The SD BIOLINE *H. pylori* rapid antibody test was found sensitive and specific for screening of *H. pylori* infection in Ethiopian populations. Therefore, this point-of-care test best fit our context and could be used as alternative to ELISA testing.

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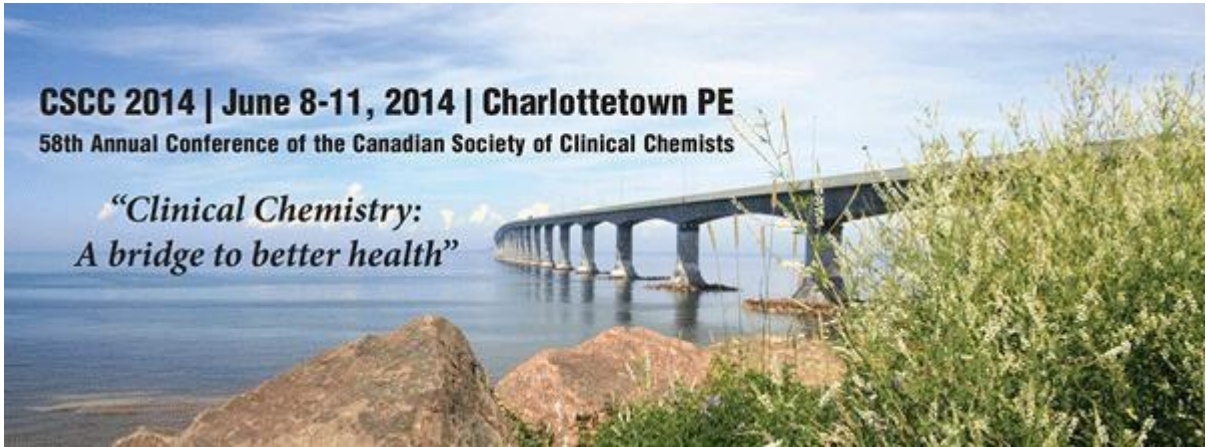
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