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DOT-ELISA and parasitological examination for diagnosis of *Schistosoma mansoni* infection in Nigeria

H. B. MAFUYAI[1], C. J. UNEKE[2*], M. O. NJOKU [3], G. CHUGA[1]

[1]Department of Zoology University of Jos. P.M.B. 2084 Jos, Nigeria; [2]Department of Medical Microbiology/Parasitology, Faculty of Clinical Medicine, Ebonyi State University, Abakaliki Nigeria, E-mail: *Unekecj @yahoo.com*; [3]GEDE AIDS/Infectious Diseases Research Institute, Abuja, Nigeria

Summary

Schistosoma mansoni infection constitutes significant economic and public health consequences. Accurate diagnosis of the infection is imperative in order to identify subjects needing care in the broadest sense. In this study the dot-ELISA and direct parasitological method were compared in the diagnosis of S. *mansoni* infection in Jos, Nigeria. Of the 106 subjects studied 64.2 % were *S. mansoni* positive serologically while only 10.4 % of them were found to have the parasite's egg in their stools. Individuals aged 20 – 29 years of both sexes had highest prevalence of 73.9 % and 21.7% by serological and parasitological diagnosis respectively. Prevalence of infection among males was 13.5 % and 7.4 % among females by parasitology. By serology the corresponding figures were 59.6 % and 68.5 % respectively. No significant difference was observed by parasitology (χ^2=4.534, P $\leq$ 0.05) and by serology (χ^2=2.00, P $\leq$ 0.05). Results advocate for serodiagnostic technique over parasitological method in *S. mansoni* surveillance and epidemiological studies.

Key words: *Schistosoma mansoni*; infection; dot-ELISA; diagnosis; prevalence

Introduction

Schistosomiasis remains one of the most prevalent parasitic infections and has a significant economic and public health consequence. It is estimated that 200 million people are infected in 74 countries of which 120 million are symptomatic and 20 million have severe disease, while 600 million people are at risk of infection (WHO, 2002). Africa is worst hit with about 85 % of the infected cases occurring in the continent. In Nigeria, up to 101 million people are at the risk of Schistosomiasis with nearly 26 million infected (Chitsulo *et al.,* 2002). Intestinal schistosomiasis caused by *Schistosoma mansoni* has been described in many parts of Nigeria and foci of hyper endemic infections have been identified (Ofoeizie *et al.,* 1996; Agbolade & Odaibo, 1996; Attah *et al.,* 2002)

S. mansoni causes an important infection of public health concern and constitutes a serious problem on the schistosomiasis control strategy in the country largely due to the logistical problems associated with its diagnosis. Due to the large day-to-day variation and the relatively small amount of faeces examined using direct parasitological methods (direct smear, formol-ether concentration, katokats slide) light infections are often missed (van Lieshout *et al.*, 2000). In addition DeVlas and Gryseels (1992) noted that even when applying the frequently used protocol of two or more stool examination per individual prevalence is still underestimated.

Following the increasing interest in the improvement of the invasive, low-sensitive and often cumbersome direct parasitological diagnosis, a more accurate diagnostic assay which is fast, gives uniform and reproducible results and which is easy to interpret is gaining more significance. Hence a number of immunological techniques have been described for the diagnosis of schistosomiasis (van Leishout *et al*., 2000; Feldmeirer & Poggensee, 1993; Tsang & Wilkins, 1997). Of these the enzyme-linked immunosorbent assay (ELISA) has been reported as very useful for shistosomiasis studies.

Recent data and information on intestinal schistosomiasis in Jos, Plateau State and other parts of central Nigeria are rather scanty and focal (Akufongwe *et al*., 1996; Egwunyenga *et al.,* 1994). These investigations however relied solely on parasitological diagnosis. The objective of this study therefore, was to compare a direct parasitological

* Corresponding author

method (formol-ether concentration) (WHO, 2003) and an immunological method (dot-ELISA) (Boctor *et al.*, 1987) for the diagnosis of *S. mansoni* infection with a view to adopting a more rapid, less invasive and reliable diagnostic procedure.

Material and Methods

Study area and population: Jos-Plateau is located in an area covering about 9 400 km^2 of the crystalline complex in Central Nigeria, its average elevation is about 1 250 m above mean sea level and has an average annual rainfall of about 1100 mm with temperature ranging from 12°C – 31°C. The two main rivers that transverse virtually the entire city are the river Dilimi and river Gada-biu. The study was carried out in the Tudun-Wada settlement in the southern part of the city. Our choice of the area was as a result of increasing reports of *Schistosoma* infections in the hospitals and clinics in the area, which indicate a near epidemic situation.
One hundred and six individuals indicated interest to participate in the study after a successful community mobilization by the research team, with the assistance of community health workers, traditional and religious leaders in the area.

Sample collection: Labeled specimen bottles for stool collection were given to individuals (whose informed consent had been obtained) a day prior to sampling. As individuals submitted their fresh stool samples the following day, 5mls of venous blood was obtained from each person by venepuncture. Demographic information with respect to age and sex was also obtained from each participant. Each blood sample was immediately transferred into a vacutainer; serum was later derived from each in the laboratory and stored at -20°C until assayed. Stool samples, which could not be studied immediately, were preserved in 5 % formalin until they were examined. The formol-ether concentration technique (WHO, 2003) was used for the parasitological diagnosis while the dot-ELISA was used for the serological assay.

Dot-ELISA: This was performed as outlined by Pappas *et al.* (1984) and Madwar and Hassan (1989). Briefly, nitrocellulose strips (1.5 X l.5 cm) were coded for positive controls, negative control and test samples. The soluble egg antigen (SEA) used was prepared by the homogenization of *S. mansoni* eggs in phosphate buffered saline (PBS) as described previously (Da Silva & Ferri, 1968; Boros & Warren, 1970; Dunne *et al.*, 1981), the supernatant fluid obtained after the homogenization constituted the SEA. The strips were dotted each with 5 µl of the SEA and allowed to dry for 30 minutes. The dotted strips were then immersed in bovine serum albumin (BSA) solution for 2 hours at room temperature to block free sites. Strips were then washed 3 times using 0.05 % PBS - Tween 20, and immersed into 3 ml of a 1:100 dilution test serum and incubated overnight at 4°C. The strips were washed 3 times using 0.05 % PBS - Tween 20, and then incubated for 2 hours at 4°C in a 1:1000 dilution of anti-human IgG conjugated to peroxidase (FIOCRUZ). Reactions were demonstrated by immersion of the strips in a freshly prepared solution of diaminobenzidine and hydrogen peroxide. Colour development occurred in 5 minutes and reactions stopped with distilled water.

Results

Of the 106 individuals screened 68 (64.2 %, 95 % CI., 55.1 – 73.3 %) were found to have antibodies to *S .mansoni* by

Table 1. Age and sex related *S. mansoni* prevalence by stool analysis (parasitology) in Jos- Nigeria

Age (years)	Male		Female		Overall total		95% Confidence interval
	Number examined	Number (%) infected	Number examined	Number (%) infected	Number examined	Number (%) infected	
0 – 9	14	2 (14.3)	8	0 (0.0)	22	2 (9.1)	2.9 – 21.1
10 – 19	20	2 (10.0)	14	1 (7.1)	34	3 (8.8)	0.7 – 18.3
20 – 29	12	3 (25.0)	11	2 (18.2)	23	5 (21.7)	4.8 – 38.5
>30	6	0 (0.0)	21	1 (4.8)	27	1 (3.7)	3.4 – 10.8
Total	52	7 (13.5)	54	4 (7.4)	106	11 (10.4)	4.6 – 16.2

Table 2. Age and sex related. *S mansoni* prevalence by dot- ELISA (serology) in Jos Nigeria

Afe (years)	Male		Female		Overall total		95% Confidence interval
	Number examined	Number (%) infected	Number examined	Number (%) infected	Number examined	Number (%) infected	
0 – 9	14	9 (64.3)	8	5 (62.5)	22	14 (63.6)	43.5 – 83.7
10 – 19	20	8 (40.0)	14	11 (78.6)	34	19 (55.9)	39.2 – 72.6
20 – 29	12	9 (75.0)	11	8 (72.7)	23	17 (73.9)	56.0 – 91.8
>30	6	5 (83.3)	21	13 (64.9)	27	18 (66.7)	47.4 – 86.0
Total	52	31 (59.6)	54	37 (68.5)	106	68 (64.2)	55.1 – 73.3

Dot-ELISA while 11 (10.4 %, 95 % CI., 4.6 – 16.2 %) of them had the parasite eggs in their stool samples (Tables 1 and 2). Individuals of age group 20 – 29 years of both sexes had the highest prevalence of 73.9 % (95 % CI., 56.0 – 91.8 %) and 21.7 % (95 % CI., 4.8 – 38.5 %) by serological and parasitological diagnosis respectively. The prevalence of the infection amongst the males was 13.5 % (95 % CI., 4.2 – 22.8 %) and 7.4 % (95 % CI., 0.4 – 14.4 %) amongst the females by parasitological analysis (Table 1), there was no statistically significant difference in the association between sex and infection by parasitological diagnosis (χ^2=1.03, P < 0.05, df=1). The corresponding figures by dot-ELISA for the males and females were 59.6 % (95 % CI., 46.3 – 79.2 %) and 68.5 % (95 % CI., 56.1 – 80.9 %) respectively (Table 2), statistical analysis also indicated no significant difference in the association between sex and infection by the dot ELISA (χ^2=0.95, P < 0.05, df=1). Parasitologically the least infection (3.7 %, 95 % CI., 3.4 – 10.8 %) was observed among individuals who were 30 years old and above while the serological analysis indicated the least infection amongst those that were 10 – 19 years old (55.9 %, 95 % CI., 39.2 – 72.6 %). The range of prevalence of infection for the various age categories was from 55.9 % – 73.9 % by dot- ELISA, and 3.7 % – 21.7 % by stool analysis. Statistical test showed no significant difference in the association of age and infection parasitologically (χ^2=4.53, P < 0.05, df=3) and serologically (χ^2=2.00, P < 0.05, df=3).

Discussion

In this study we obtained a serological and parasitological prevalence of 64.2 % and 10.4 % respectively. This wide disparity between the two methods was noted in previous reports (Boctor *et al.*, 1987; WHO, 1998) and is in close agreement with findings in Libya (Gabriel *et al.*, 1985) where a 6.9 % parasitological prevalence and as high as 33.4 % seroprevalence of *S. mansoni* infection were obtained. It is established that the diagnosis of an active *S. mansoni* infection is relatively easy by parasitological methods, if subjects harbor high worm loads, which is typically accompanied by high egg excretion. But when infections are not intense, the demonstration of eggs in stools is reportedly very difficult (van Leishout *et al.,* 2000) hence it is most likely that the parasitological method we used did not indicate the true *S. mansoni* prevalence of the community, as light and early infections may have been missed. Consequently we have reasons to rely more on the result of the serological assay since it has been shown to be highly sensitive and capable of demonstrating antibodies in light infections and shortly after exposure (van Leishout *et al.,* 2000; Tsang & Wilkins, 1997).

The efficiency of the dot-ELISA have been compared with that of other serological tests for *S. mansoni* diagnosis by a number of workers. Rabello *et al.* (1992), compared the efficiency of ELISA, dot-ELISA, and dot-DIA in *S. mansoni* diagnosis and observed a high level of agreement between the methods tested as follows: ELISA x dot-ELISA: 95.1 %, ELISA x dot-DIA: 92.7 % and dot-ELISA x dot-DIA: 97.6 %. In another related study, Rabello *et al*. (1993) also observed that the efficiency for *S. mansoni* diagnosis was 92.7 %, 90.0 % for ELISA, dot-ELISA and dot-DIA, respectively and concluded that dipstick dot-ELISA and dot-DIA are reliable cheap and simple methods for the serological differentiation of acute and chronic schistosomiasis. In a more recent study Van Gool *et al.* (2002) reported that combined use of ELISA and worm antigen/indirect haemaglutination (WA/IHA) gave sensitivities of 100 % for *S. mansoni*, *S. haematobium*, and *S. mansoni* and *S. haematobium* combined. However inspite of the proven efficiency of other serological test Pinto *et al.* (1995) had maintained that dot-ELISA can be used for the detection of specific antibodies against *S. mansoni* in sera from suspected patients or in epidemiological studies and, with further purification of egg antigen and larger samples, IgM Dot-ELISA could be a possible tool for rough estimates of parasite burden in epidemiological studies.

Serological evidence indicated that the *S. mansoni* infection was more prevalent among the females than the males (68.5 % Vs 59.6 %), this was contrary to the results from the parasitological analysis (7.4 % Vs 13.5 %). However evidence from epidemiological assessment of schistosomiasis in the study area showed that contact with cercariae infested waters which run through the community, is higher among the females. Activities such as washing, irrigation and fetching water from infested rivers for daily routine household chores expose the women (majority of whom were house wives) more to the infection than their male counterparts who were mainly civil servants and artisans.

In addition, this water contact activity was more common amongst individuals of age group 20 – 29 years accounting for the higher frequency of the infection amongst them. This is consistent with a previous report in northern Tanzania by Pogensee *et al.* (2002), which indicated the highest seroprevalence of 65 % among individuals of age group 20 – 30 years.

From the foregoing, our results support the superiority of immunological assay over parasitological techniques in the estimation of true prevalence values. In planning prevention and control approaches, serological screening of residents may therefore offer best methods as it would identify all infected population for chemotherapy, which reduces the prevalence and the disease burden (Rodrigo Correa *et al*., 2000). Although false positive results are possible with serological assays, however, treatable false positives are of better advantage to the individuals and communities than untreated or missed infections.

The less invasive immunological assays such as the dot-ELISA used in this study has added advantage over the rigorous stool examination method especially with the ease of handling large number of samples for epidemiological studies. Also interpretation of results is easy and requires neither special equipment nor high level of technical expertise as positive results are interpreted visually as colored spots on the white nitrocellulose strips (Pappas *et al*., 1984). We conclude therefore that these advantages out-

weigh any of the parasitological methods both in routine analysis and research purposes in *Schistosoma* related infections.

Despite these advantages the dot-ELISA may not address one of the crucial factors in the parasitological diagnosis of S. *mansoni,* which is the determination of the intensity of infection (Feldmmeier and Poggensee, 1993). The unavailability of dot-ELISA in most laboratories in most parts of the developing world as a result of its high cost is another major draw back. In addition, some degree of cross reactivity with other helminth infections, persisting elevated titers, post-treatment effects, and prolonged immunological response of the host (Tsang & Wilkins 1997), will continue to call for the research needs of appropriate diagnostic tools for the assessment of indirect schistosomiasis disease markers.

In conclusion, further studies are advocated to evaluate other serological tools in the diagnosis of *S. mansoni* infection especially in epidemiological surveillance of areas known to be endemic for intestinal schistosomiasis in this part of the globe. The feasibility, sensitivity, and rapidity of such tests need be determine to prove their cost effectiveness for the resource-scarce endemic rural areas of the tropics.

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