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Concurrent infections and neutrophil phagocytic function in Nigerians with urinary schistosomiasis

O.G. Arinola, and L.S. Salimonu
Department of Chemical Pathology, College of Medicine, University of Ibadan, Ibadan,Nigeria

Summary
Bacteria infections, parasitic infections and neutrophil phagocytic function were assessed in 60 urinary schistosomiasis (USS) subjects and 36 healthy controls. Only *Salmonella* was isolated from the blood samples of few USS subjects (3%) and controls (1%). The species of bacteria detected in the urine of USS and control subjects are *Escherichia coli, Salmonella, Staphylococcus* aureus, *Streptococcus faecalis* and *Klebsiella*. There was no significant difference in the proportion of the USS subjects with bacteriuria compared with the controls (X^2 = 0.20, *P>0.20)*. Higher proportions of USS subjects compared with the controls were concurrently infected with *Ascaris lumbricoides*, hookworms, *Giardia lambila* and *Taenia solium*. In contrast, significantly higher proportion of the control subjects were concurrently infected with *Plasmodium falciparum* and *Schistosoma mansoni*. There was no significant reduction in neutrophil phagocytic function of USS subjects compared with the controls *(P>0.49)*. This study suggests that S. haematobium protects its host from certain blood dwelling parasites and that Nigeria USS subjects expressed adequate neutrophil phagocytic function. These may explain the absence of clinical manifestations of bacterial and viral infections in these subjects.

Keywords: *Infection, Phagocytosis, Schistosomiasis, Neutrophil*

Résumé
Les infections bacteriennes, infections parasitaire et la fonction phagocytaire des neutrophiles a ete evalué chez 60 sujets presentant une Shistosomiase Urinaire (USS) et 36 sujets bien portant considere comme controles. Seule la Salmonella a ete isolé des specimens sanguins d'un petit nombre de sujet a USS (3%) et des controles (1%). Les especes des bacteries detecté dans les urines des USS et des conroles sont *Escherichia Coli, Salmonella, Staphylococcus aureus, Streptococcus faecalis*, et les *klebsiella*. Il n'y a pas eu de differences significative dans la proportion des sujets a USS avec la bacteriuria compare a ceux des controlles (X^2 = 0.20, *P* 0.020). Une forte proportion des sujets a USS compares aux controles ont ete infecte de maniere concourante avec les *Ascaris lumbricoides*, les tenias, *Giardia lambdia* et du *Taenia sodium*. Par contre, une proportion significativement forte des controles etaient infecte avec du *Plasmodium falciparum* et du *Shistosma mansoni*. Il n'y a pas eu une reduction significative de la fonction phagocitaire des neutrophiles compare aux controles (*P* 0.49). Cette etude suggere que le *S. haematobium* protege son hote contre certains parasites sanguin, et que certains sujets Nigerian à USS experiment une fonction phagocitaire des neutrophiles adequate. Ceci pourrait exprimer l'absence de manifestation Clinque des infections bacterienes et virale chez ces patients.

Correspondence: Dr. O.G. Arinola, Immunology Unit, Department of Chemical Pathology, College of Medicine, University of Ibadan, Ibadan, Nigeria.

Introduction

The frequent association of bacteria, *Chlamydia trachomatis* and hepatitis B virus with schistosome infection and complete cure after treatment of schistosomiasis have long been recognised [1-5]. The spectrum of bacteria present in the urine of schistosomiasis subjects are *Klebsiella, Escherichia coli, Proteus, Shigella* and *Salmonella* [3]. No literature relating *S. haematobium* infection with other pathogen/ parasite was encountered.

Reduced resistance to bacterial infection and viral infection in chronic human and experimental schistosomiasis has been explained [2,3,5]. Among the immunological based reasons are: modification of structure and dynamic characteristics of bacteria by schistosome antigens [6,] reduced phagocytic activity of reticulo-endothelial system [7], high urinary excretion of IgA and depressed serum level of complement [8] and formation of large amount of immunosuppressive circulating immune complexes [9]. Wellhausen and Boros [10] proposed decreased bactericidal activity of macrophages obtained from urinary schistosomiasis subjects while reduced production of beta-glucuronidase and superoxide was suggested by Olds and Ellner [11].

The present study established an intercurrent relationship between *S. haematobium* infection with other parasites/pathogens in the same host; also the likely mechanism of the observed result is discussed.

Materials and methods.

Urinary schistosomisais (USS) subjects.

They were 60 school children attending St. John's Primary School (SJPS) Mokola, Ibadan, Nigeria. They were diagnosed by identification of terminal spined eggs of *S. haematobium* in urine sediments following centrifugation at 1500 – 200 rpm for 5 minutes. *Schistosoma* infection was categorised into light and heavy-infection based on the number of eggs counted per 10 ml urine as described by W.H.O. [12]. Eggs count between 1 and 49 per/10 ml urine was categorised as light infection while egg number of 50 and above was taken as heavy infection.

Controls

A total of 36 *S. haematobium* free children from the same

school (SJPS) and classroom were considered as controls. They were apparently healthy children matched for age, sex and socioeconomic status with USS subjects.

Parasitological investigations

From both USS and control subjects, faecal samples were collected with white screw-capped plastic ice cups. The sample containers were marked and the subjects were instructed to provide their early morning faecal specimens. These were collected between 8.00 and 10.00 hours. Sample specimens were flooded with 5% formalin on collection. About 2 g of the faecal specimen was thoroughly mixed with normal saline until a uniform suspension was obtained. This was transferred into a centrifuge tube after straining through a mesh sieve to remove debris. It was centrifuged at 3000 rpm for 3 minutes. The supernatant was discarded. Drops of the sediment were placed on a microscope slide and stained with Lugol iodine. This was covered with coverlips and examined through 40 x –400 objective lens.

A thick blood film was prepared on a glass slide from a finger prick (using lancet) to investigate the status of *Plasmodium falciparum* infection in all the subjects. This was stained with Giemsa before it was viewed with a compound microscope under oil immersion lens. Parasite number of between 1 and 10 in every high power field was considered heavy infection (+++).

Microfilaria and *Trypanosoma* were also examined in thin and thick film on microscope slides stained with Giemsa and viewed with low power objective lens before confirmation with high power objective lens.

Bacteriuria

Mid-stream urine samples collected in a sterile container from USS and control subjects were plated on both blood sugar and CLED (Cysteine Lactose Electrolyte Deficient) media. These cultured plates were incubated overnight at 37 °C. Only cultures with bacterial count of greater than 10^5 colonies/ml of urine from two consecutive tests were considered by routine methods following the scheme of Cowan and Steel [13].

Bacteremia

Blood samples collected by venepunctre were delivered into blood agar broth (liquiod) in a sterile glass bottle. This was well but gently mixed and incubated at 37 °C for 7 days. The set up was checked everyday for turbidity. The few turbid ones were transferred to a selective medium for growth and identification of colony as described by Cowan and Steel [13].

Neutrophil candidacidal assay.

The abilities of neutrophils to kill *Candida albicans* were determined by the method of Lehrer and Cline [14]. A saline-wash concentrated suspension of a 24-hour culture of *C. albicans* was made in Hank solution. This was adjusted to 5 x 10^6 cells/ml of Hanks solution and viability of the cells was confirmed to be above 95% by the trypan-blue dye-exclusion method. To a mixture of 0.25 ml Hank solution, 0.25 ml autologuous plasma and 0.25 ml of 5 x 10^6/ml suspension of *Candida* in Hank were added to 0. 25 ml of 5 x 10^6/ml neutrophil suspension in Hank solution. A similar set up was made for the control tube except that neutrophil suspension was omitted. The tubes containing the mixture were incubated at 37 °C for 1 hour with shaking every 15 minutes. At the end of this period, 0.25 ml of 2.5% sodium desoxycholate as added to each mixture. This lysis the neutrophil, but not the *Candida.* Four (4) ml of 0.01% methylene blue was then added to strain any dead *Candida* cell and the tube centrifuged at 1500 g for 10 minutes at 4 °C. Supernatant methylene blue was carefully removed leaving about 0.5 ml to resuspend the organisms. The percentages of dead *Candida* (stained cells) were determined using the improved Neubauer counting chamber.

Total and differential, white blood cell counts:

These was done as described in a standard haematology textbook using Tuerk solution and Leishman stain [15].

Statistical methods

The data generated from this study were analysed for means, standard deviations, Students t-test and one-way analysis of variance.

Results

Table 1: Prevalence of parastic infections in urinary schistosomiasis (USS) subjects and USS free controls

Parasite species	Subjects: USS subjects (n = 60)	Subjects: Controls (n = 36)	X^2; P-value
Plasmodium falciprum	7(12%)	10 (28%)	4.01, < 0.05
Microfilarae	1 (12%)	2 (6%)	0.02, > 0.20
Trypanosoma brucei	Nil	Nil	Nil
Ascaris lumbricoides	17 (28%)	8 (22%)	0.44, > 0.20
Hookworms	6 (10%)	2 (6%)	0.58, > 0.20
S. stercoralis	2 (3%)	1 (3%)	0.02, > 0.20
S. mansoni	3 (5%)	7 (19%)	5.01 < 0.025
Taenia sp.	7 (12%)	2 (6%)	0.19, > 0.20
Entamoeba histolytica	5 (8%)	3 (8%)	0.00, > 0.20
Giardia lambila	1 (2%)	Nil	0.61, > 0.20

Table 1 shows that *Ascaris lumbriodies, hookworms, Taenia solium* and *Giardia lamblia* were more prevalent among USS subjects, though the prevalence of these parasites in USS subjects compared with *S. haematobium* free subjects was not significant ($P>0.20$ in each case). Also in Table 1, *S. mansoni* and heavy infection of *Plasmodium falciparum* were more prevalent in *S. haematobium free children than subjects with S. haematobium* infection ($P<0.025$; $P<0.05$)

Table 2: Incidence of bacteria species in the urine sand blood sample of school children with or without schistosomiasis.

Bacteria species	Subjects: USS subjects (n = 60	Controls (n = 36)	X^2 P- value
(a) In the urine			
E Coli	4 (7%)	1 (3%)	0.20, > 0.20
S aeureus	3 (5%)	2 (6%)	0.20, > 0.20
S faecalis	3 (5%)	1 (3%)	0.28, > 0.20
Klebsiella sp.	2 (3%)	1 (3%)	0.03, > 0.20
Salmonella sp.	6 (10%)	3 (8%)	0.07, > 0.20
Total	18 (30%)	8 (22%)	0.28, > 0.20
(b) In the Blood Salmonella	3 (5%)	1 (3%)	0.28, > 0.20

Table 2 shows that five general of bacteria (*E. coli, S. aureus, S. faecalis, Salmonella* and *Klebsiella*) were isolated in the urine of USS and control subjects, with *Salmonella* occuring most among the two subjects groups. Though different proportions of USS subjects and control subjects had bacteria in their urine samples, these differences were however not significant *(P > 0.20)* Only *Salmonella* was isolated from the blood of few USS subjects (5%) and the controls (3%).

Table 3: Comparison of total white blood cell counts and neutrophils* in USS severity group and the controls.

WBC $X10^9$	USS severity groups: Light (n = 24)	Heavy (n = 36)	Control (n = 36)	*P* values
Total White Blood cell	7.63 ± 2.69	9.54 ± 2.44	5.52 ± 2.14	<0.001
Neutrophil	3.81 ± 0.47	4.13 ± 0.59	3.81 ± 0.49	<0 033

* *Values are mean ± I.S.D.*
** *One-way analysis of variance (P <0.05 is significant)*
= *Significantly different from control group P< 0.05 (t-test)*
\+ = *Significantly different from lightly infected group, P <0.05 (t= test).*

Table 3 shows that total white blood count was significantly higher in heavily infected subjects compared with the controls *(P < 0.01)* and also when heavily subjects were compared with the lightly infected group *(P < 0.01)*. Neutrophil count was significantly increased in heavily infected subjects compared with the lightly infected subjects *(P < 0 05)*

As shown in Table 4, the candidacidal activity of the neutrophils was not significantly different in the controls compared with lightly infected subjects *(P < 0.20)*, or heavy USS subjects *(P < 0.10)*.

Table 4: Neutrophils phagocytic activity* in USS subjects and the controls.

Subjects	Candidacidal activity (%)
Controls (n = 36)	27 .88 ± 5.44
Light infection (n = 36)	27. 06 ± 6.09
Heavy infection (n = 24)	26.13 ± 4.84
P**	> 0.490

* *Values are mean ± 1 S.D*
** *One-way analysis of variance (P-value) 0.05 is significant)*

Discussion

The occurrence of *Ascaris lumbricoides, hookworms, taenia solium* and *Giardia lambia* have been found in areas where there is laxity in personal hygiene [16, 17, 18, 19]. This in one of the disposing factors to *S. haematobium* infection, therefore relatively higher occurrence of these parasites in USS subjects is not a surprise.

The absence of heavy (+++) *Plasmodium falciparum* infections among *S. haematobium* infected subjects may not be easily explained, but the following reasons may be speculated. Adult worms and eggs of *S. haematobium* are blood dwellers on which red blood cells adhere, to result in haemolysis [20]. The purpose of this mechanism was suggested by Farid et al. [9] to prevent sloughing of the bladder wall by ova deposited by schistosome adults. These authors also suggested that schistosome adhered blood cells release their haemoglobin which precipitates neutralises the offensive secretion of the miracidia, schistosomes toxin and enzymes. *Plasmodium* - infected RBC are fragile and lyse easily [21]. Adherence of *Plasmodium* – infected RBCs to *S. haematobium* adult worms or eggs may aggravate their lyses. This will cause reduction in number of *Plasmodium* – infected RBC and exposure of *Plasmodium* from lysed RBC to offensive immune system of the host [20].

The phenomenon of concomitant immunity may be used to explain the significantly reduced occurrence of *S. mansoni* infection in *S. haematobium* – infected subjects. It is likely that stimulating antigen on *S. haematobium* is also present in *S. mansoni* or that the protective factor produced against *S. haematobium* do cross react against *S. mansoni*. This corroborates the finding of McLaren [22]. He concluded that penetration of cercariae into human host stimulates immune responses which protects against reinfection by schistosome larva [22].

Trypanosoma brucei was not detected in these school children, this may be due to lack of an intermediate host (*Glossina sp.)* of this parasite in this area.

The results presented in Table 2 show that only *Salmonella sp.* was detected in the urine and blood of USS subjects and the controls. Other bacteria (*E. coli, Staphylococcus, aureus, Streptococcus faecalis* and *Klebsiella)* were detected in the urine samples of both USS and control subjects. These are enterobacteriaocae which are usually contacted through faecal contamination and in areas where waste disposal system is inadequate [17,18]. These conditions favour the spread of *S. haematobium* and *S. mansoni* [16,18, 19]. To this end, bacteria might have entered the circulation of unsuspecting host when the skin is broken by penetrating cercariae or by bacteria-coated cercariae since both cercariae and bacteria dwell within the same micro environment. Bacteria in the circulation of USS subjects are ingested by phagocytes in USS subjects at the same rate as in *S. haematobium*–free individuals. This is indicated by our result of neutrophil candidacidal (phagocytic) function.

Neutrophils form the first line of immunological defence and are important in the removal of damaged tissues [23]. However, primary defects of neutrophil function are rare but secondary defects of neutrophil function are the commonest, particularly in severe infection [24]. Data from this study showed that neutrophils from USS subjects showed a normal, though slight decreased capacity to ingest *Candida albicans*. Literature concerning neutrophil phagocytic function to non-schistosome antigen(s) was not encountered, but related work showed that macrophages from chronically infected USS subjects displayed a diminished bactericidal activity [25].

Two possibilities may be speculated to explain our observation. It is possible that the neutrophils involved in phagocytosis of *C. albicans* are immature. In support of this hypothesis is the detection of significantly increased neutrophil number in USS subjects compared with the controls. Most of these may be newly formed.

Alternatively, the reduced candidacidal activity of neutrophils could have been due to the binding of soluble antigen from *Schistosoma* parasites or soluble immune complexes to membrane receptors, therefore impeding effective binding and internalisation of *C. albicans*. Massive production of antigens [26,27] and high concentration of soluble immune complexes is common to *Schistosome* infection [28,29,33]. This speculation corroborates the report of Capron and Capron [30] and Aurialt *et al.* [31] who detected large amounts of blocking antibodies and immune complexes in the serum and on the surfaces of peritoneal cells collected from animals with chronic schistosomiasis.

This study shows that cell-mediated immunity in these USS subjects is adequate and this may explain lack of clinical manifestation of bacterial or viral infections in these subjects. In support of our previous study [32], USS in these subjects is in the acute stage, though susceptibility to concurrent infection in eminent if *S. haematobium* infection in these children is allowed to advance into the chronic stage.

It may therefore be concluded that the presence of *S. haematobium* parasite reduces concurrent infection with *S. mansoni* or *Plasmodium* sp. and this may have both immunological or haematological base.

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