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SERA FROM NIGERIAN CHILDREN WITH GENITOURINARY SCHISTOSOMIASIS HAVING IMMUNE COMPLEXES AND HEAT LABILE LEUCOCYTE MIGRATION INHIBITORY FACTORS WITHOUT IMPAIRED CELLULAR IMMUNITY

NCD Ukwandu[a]* and OPG Nmorsi[b]

[a]Department of Medical Microbiology, Faculty of Clinical Sciences, College of Medicine, Edo State (Ambrose Alli) University, PMB 14, Ekpoma, Nigeria

[b]Department of Zoology, Faculty of Natural Sciences, Edo State (Ambrose Alli) University, PMB 14, Ekpoma, Nigeria

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There was a significant increase in the rate of synthesis of both albumin and globulin as a result of good adaptive mechanism which prevent hypoproteinemia in children with genitourinary schistosomiasis from endemic areas in Edo and Delta States, South Eastern Nigeria. Single radial immunodiffusion method and the Mantoux test were used to evaluate serum acute phase proteins and delayed hypersensitivity skin assay. While the Nytrel filter method of World Health Organization was employed in the counting of *Schistosoma haematobium* ova and the serum inhibitory factor to leucocyte migration was determined in accordance with WHO specifications with modifications in the preparation of the two antigens and a mitogen - BCG, IMV and PPD. Results were interpreted statistically using spearman's coefficient of correlation and regression analysis. Complement factors present in circulating soluble immune complex and complement dependant cell mediated and killed schistosomula. C_4 decreased with increase in number of *S.haematobium* eggs while C_3 (C_3C) products increased with severity of infection. There was an acute phase response to tissue damage by all stages of schistosomes and inflammatory response of immune competent cells against schistosome antigens from eggs and worm organs, which resulted to increase in transferrin. It was suggested that the heat labile leucocyte migration inhibitory factors were present in the sera of *S.haematobium* infected children, and there was a reduced negative skin response to tuberculin antigen in the infected children. These facts establish the possession of adequate functioning of the cytotoxic $CD8^+$ T lymplocytes in the infected children. The heat labile migration inhibitory factors are products of immune complexes which when activated by complements result to patechial haemorrhagic manifestations. Leucopenia, atypical lymphocytes and plasma cells were observed in the blood which are characteristics of children who have experienced previous attack of *S. haematobium* infection.

Key words: Genitourinary schistosomiasis, Immune-complex, Leucocyte-migration, Cellular immunity.

Introduction

Most studies on *Schistosoma haematobium* are on the level of epidemiology, pathology, biology, control and prevention. However, immunology of genitourinary schistosomiasis in relation to urinary egg count among Nigeria school children has not been fully studied. Available information on the immunological studies of urinary schistosomiasis by Lucas and Boros (1992) and Newar *et al* (1992), on experimental animals are inconsistent and focused attention on a few indices.

The belief that immunity is a major factor controlling the prevalence and intensity of schistosomiasis in men is a deep seated one. It seems impossible to most observer that subjects in endemic areas who are exposed to infected waters are not constantly re-infected. Furthermore, there is a continuous trickle of anecdotal evidence of the infectivity of the parasite supplied by case reports of individual from non-endemic areas who became infected after only a short term exposure (Warren 1973).

During infection patients undergo immunologic modulation, which results to decrease inflammation around the eggs, and this allows patients to survive up to chronic stage of infection (Boros *et al* 1975). The evasion of penetrating schistosomula from the destruction of eosinophils and non-specific antibody of the host activates the macrophage, as further defensive machanism (Olds and Ellner 1984). The importance of macrophage in schistosome infection derides from increased secretion of granule-poietic colony stimulation factor (CSF) from monocyte-macrophage and lymphocytes (Bolin and Robinson 1977; Verman *et al* 1979). Schistosomiasis is associated with a significant decrease in the bacteria-phagocytic

*Author for correspondence

Table 1
Mean serum levels of total proteins albumin and globulin in schistosomiasis infected children and control

Subjects	No. examined	Total proteins (g/100ml)	Albumin (g/100ml)	Globulin (g/100ml)
Control	50	7.33 + 0.62	2.96 + 0.38	2.60 + 0.45
Lightly infected children (<50 eggs/10ml urine)	75	7.02 + 0.41	2.54 + 0.15	2.48 + 0.42
Heavily infected children (>50 eggs/10ml urine)	75	5.49 + 0.61	2.15 + 0.32	1.96 + 0.43
Control and heavily infected compared		2.40 < 0.02	1.50 < 0.01	0.40 < 0.30
Control and heavily infected compared		3.10 < 0.01	9.60 < 0.02	1.40 > 0.02
Lightly infected and heavily infected compared		0.60 < 0.02	1.00 < 0.03	2.30 < 0.02

index of macrophages due to reduced proportion of cells engaged in phagocytosis (Wellhansen and Boros 1981). The objective of this study was to investigate the immunobiological effects of *S. haematobium* on children with chronic genitourinary schistosomiasis.

Materials and Methods

Two hundred primary school children from *S. haematobium* endemic areas were enrolled in the study. These children were 5-15 years old, comprising of 75 lightly and heavily infected children. Their sexes were in equal ratio in each category.

Controls. There were 50 primary school children whose ages were within 5-15 years old and were sex matched with the infected children. These were all apparently healthy and immune from *S. haematobium* or any other blood helminth infection.

Blood collection. Consent were sought from both parents and teachers before blood was drawn from each of their children. About 10ml of venepuncture blood was drawn from each child and 5ml placed on clean plane glass container till refraction for serum operation.

Exclusion of other parasites. Plasmodia, trypanosomes and blood micro filarial agents were all included by thick smear stained with Geimsa of pH 7.2. This was examined under x40 and later confirmed under x100 objective lens using light microscope.

Serum acute phase proteins. Serum acute phase protein like the C_4 ,C_3C, transferrin, C-reactive proteins, α-2-macroglobulin, haptoglobulin and caeruloplasmin were determined and evaluated using single radial immunodiffusion method of Mancini *et al* (1965).

*Counting of S.haematobium eggs.*Highly turbid or haematuric urine sample were diluted appropriately with normal saline or phosphate buffered saline of pH 7.2 before counting of *S. haematobium* eggs. Nytrel filter was used to count the eggs microscopically in accordance with World Health Organization (WHO 1983).

Serum inhibitory factor to leucocyte migration. The serum inhibitory factor was based on the Hudson and Hay (1976), with slight modifications by including both the sera of test and control children in the solution used in the preparation of the antigens and mitogen (BCG, IMV and PPD).

The walls in migration chamber contain cut capillary tubes topped with medium or antigen - sera medium solutions. These were done in duplicates. About one volume of 1 in 50 of each antigen prepared was mixed with equal volume of 1 in 50 of both test and control sera each. Then the percentage migration due to the presence of antigens and sera were calculated by using:

$$MIS = \frac{Ms}{Mc} \times \frac{100}{1}$$

Where MIS is the migration index in the presence of antigen and serum, Ms is the sera of the migration in the presence of antigen and serum solution and Mc is the sera of migration in medium (15% foetal calf serum).

Delayed hypersensitivity skin assay using Mantoux test. The administration of Mantoux test was through injection of 0.1ml (5 tuberculin units-TU) of phenol-preserved and Tween-80 stabilized purified protein derivative (PPD) using a plastic insulin syringe and gauge 27 steel needle. The injection site was the middle third of the anteromedial aspects of the left forearm. Each vial of 10 dose of PPD was used up

Table 2
Distribution of acute phase proteins amongst children with urinary schistosomiasis

Subjects	No. examined	C_3 (mg/100ml)	C_4 (mg/100ml)	c- reactive proteins (mg/100ml)	Caeruloplasm in (mg/100ml)	α-2-macro globulin (mg/100ml)	Haptoglobul in (mg/100ml)	Transferrin (mg/100ml)
Control	50	98.1±6.5	19.6±6.2	1.2±0.1	30.6±5.2	23.7±0.1	70.1±42.1	197.2±31.0
Lightly infected children (1-50 eggs/10ml urine)	75	105±11.2	25±12.1	3.11±1.1	33.4±6.2	25.2±1.2	88.6±58.2	182±9.6
Heavily infected children (>50 eggs/10ml urine)	75	110±7.5	12.6± 6.3	3.15±0.2	32.5±5.4	26.5±4.3	94.5±60.2	172±6.5
Control and lightly infected compared		3.6<0.02	0.30>0.02	6.0 <0.01	1.4 >0.02	2.1 <0.02	0.7 >0.1	1.2 >0.03
Control and heavily infected compared		6.2<0.02	4.2<0.01	7.3 > 0.01	0.9 >0.02	2.0 <0.01	1.2 >0.1	1.9 <0.01
Lightly infected and heavily infected compared		1.0>0.01	2.4<0.02	2.4 <0.02	0.1 >0.01	1.4 >0.1	1.2 >0.01	1.6 >0.2

before the next was opened. The result was read at the third day after administering the injection and children were instructed not to scratch the site/ point of the injection. The results were read as follows: an induration measuring zero to 4mm was interpreted as negative, 5mm to 9mm as intermediate and above 10mm as positive.

Statistical methods. Results were interpreted using Student's t-test, Chi-square (X^2) test, Spearman's Coefficient of Correlation and Regression analysis.

Results and Discussion

Table 1 shows the mean serum levels of total proteins, albumin and globulin in genitourinary schistosomiasis infected children and control. The mean value of total proteins reduced significantly among the heavily infected children and lightly infected children when compared with the control. This was not the case when heavily and lightly infected were compared. There was a marked reduction of mean albumin level comparing lightly infected with control, lightly infected with heavily infected and control with heavily infected. The mean serum globulin level was not significantly reduced, comparing control with lightly infected but there was a significant reduction when globulin level in heavily infected was compared with control and lightly infected. There was no significant correlation between total protein, albumin and globulin with egg count ($r = 2.1$, $p > 0.01$; $r = 0.4$, $p > 0.02$ and $r = 2.0$, $p > 0.20$) in that order.

Table 2 shows the distribution of acute phase proteins amongst children with genitourinary schistosomiasis. Significant difference existed between the infected children and the control when the levels of C_3C, C-reactive protein and α-2-macroglobulin were compared. Significant difference also existed between the lightly infected when the mean values of C_4 and C- reaction protein were compared. C- reactive protein and α-2-macroglobulin showed a marked correlation with eggs counted ($r = -0.16$, $p<0.005$, $r = -0.15$, $p < 0.005$), respectively. Both C-reactive protein and haptoglobulin were detected in 26 (52.0%) and 12 (24.0%), respectively from the control.

Table 3 shows the leucocyte migration index from control, lightly and heavily infected children, vis-à-vis the serum inhibitory substances, using tuberculin purified protein derivative antigen, with either pooled sera from control, lightly and heavily infected, or heat inactivated pooled sera from heavily infected. Migration index of leucocyte from control was significantly decreased by the addition of sera of children with either lightly or heavily infected. The addition of sera from heavily infected children showed highest migration index. The migration index from control was highest in the presence of inactivated pooled sera. Only the sera from heavily infected children imparted a marked reduction in the mean migration index of leucocytes from lightly infected, and mean migration index of leucocytes from heavily infected.

Table 4 shows the result of purified protein derivative (PPD-Mantoux skin test) on both the control and the infected children- both treated and untreated. The result revealed marked increase in mean diameter of induration - Mantoux skin test reaction, in the heavily infected when compared with the control and in the lightly infected, when compared with the heavily infected before and after treatment. Out of 55 untreated and 70 treated infected children, 11 and 13 chil-

Table 3
The leucocyte migration index from control, lightly and heavily infected children compared to the serum inhibitory substances

Sources of sera	Control		Lightly infected (1-50 eggs/10ml urine)		Heavily infected (>50 eggs /10ml urine)	
	No. examined (n=50)	Mean % age migration index of leucocytes from control	No. examined (n=75)	Mean % age migration index of leucocytes from lightly infected	No. examined (n=75)	Mean % age migration index of leucocytes from heavily infected
i) Foetal calf serum	10	65.7 ± 1.20	15	61.5 ± 4.30	15	57.9 ± 6.10
ii) Sera from control children	10	60.5 ± 10.1	15	58.3 ± 8.40	15	52.3 ± 7.20
iii) Sera from lightly infected	10	54.1 ± 9.40	15	56.2 ± 9.20	15	48.2 ± 5.30
iv) Sera from heavily infected	10	42.2 ± 8.30	15	48.9 ± 10.4	15	33.1 ± 10.2
v) Heat inactivated sera from heavily infected	10	72.8 ± 5.20	15	60.1 ± 8.30	15	55.5 ± 3.40
Comparison						
i and ii		1.3 > 0.01		0.2 > 0.01		1.2 < 0.20
i and iii		2.4 > 0.02		0.4 > 0.02		1.4 < 0.01
i and iv		7.3 < 0.01		1.2 < 0.04		7.2 > 0.10
i and v		0.4 > 0.10		0.4 > 0.10		0.5 < 0.02
Ii and iii		1.2 > 0.01		1.6 > 0.01		0.2 > 0.01
Ii and iv		3.4 < 0.01		0.1 < 0.02		0.4 < 0.02
Ii and v		5.1 > 0.01		2.1 > 0.01		0.3 > 0.02
iii and iv		1.0 < 0.01		1.3 < 0.02		0.1 < 0.03
iii and v		1.2 > 0.01		1.0 > 0.01		1.1 > 0.01
iv and v		3.2 < 0.02		3.2 < 0.01		2.3 > 0.01

dren, respectively had their Mantoux skin test negative by having the diameter of their skin reaction less than 4mm (X^2 = 4.15, p> 0.01 and X^2 =2.21, P > 0.01, respectively). While 17 out of the 50 uninfected control children showed negative skin reaction. The correlation coefficient of the skin reaction diameter of Mantoux test and eggs counted using tuberculin PPD antigen showed a significant positive result (r = 0.26, P< 0.01). While the correlation coefficient of skin reaction diameter of Mantoux test and leucocyte migration index using tuberculin PPD antigen showed a negative result (r = 0.41, P> 0.02).

There was loss of protein from the urine of schistosome infected children as reported by Ukwandu *et al* (2001) yet, the mean values of the total protein in both the lightly and heavily infected children still falls within the normal reference range of (6.7-8.2)g / 100ml. This could be as a result of good adaptive mechanisms, which prevent hypoproteinemia in genitourinary schistosomiasis patients in the form of increase in the synthesis of albumin and globulin. The mean synthesis of these protein have been observed in this study to be significantly higher in the heavily infected children when compared with the lightly infected children and control. It has been established by Rasheed *et al* (1990) and Hussein *et al* (1993) that the presence of complement factors (C1q C_4 and C_3) in circulating soluble immune complexes and in complement dependent cell mediated kill schistosomula.

However, none of the studies did relate complement factors and other acute phase reactants to number of eggs of schistosoma. This study has established that C_4 decreases with increase in the number of *Schistosoma* eggs; while C_3 (C_3C) products increase with severity of genitourinary schistosomiasis. This has tallied with the works of Santoro *et al* (1980) who detected an increase in C_3d amongst heavily infected children with *S. mansoni*. A factor of complement activation by *Schistosoma* antigens was believed to have been responsible for the decreased C_4 and increased C_3C levels vis-à-vis number of eggs excreted and the release of proteinase enzymes by schistosomes. This reacted directly with complements' factor, which resulted in hypocomplementemia.

Irrespective of the severity of the disease, yet remarkable decrease in transferrin was observed when heavily infected children were compared with the control.

This observation could be due to acute phase response to tissue damage by all stages of schistosomes that affected

human. The penetration of the skin by cercariae damaged the skin, while schistosome adults adhered to red blood cells (RBC) and subsequently lysed the RBC. The lysed RBC, thus released iron which then bound with transferrin. This was then passed directly into the developing erythrocyte to form haemoglobin.

In the alternative, the observed low level tranferrin could be due to schistosome eggs which destroyed the tissues during circulation or inflammatory response of immune competent cells against schistosome antigens from eggs and worm origins. The bacterial infection was a result of low transferrin level added to reduce efficiency of phagocytic cells at the late acute and early chronic stages of schistosomiasis. This is supported by the fact that transferrin is an iron-binding protein in serum. Low level means that free iron is abundant for use by bacteria i.e. iron for their multiplication.

Haptogblobulin was observed to increase with increase in number of eggs. Haptoglobulin is known to bind free haemoglobulin which is then degraded for iron to be saved in the liver. Stites (1980) showed that a high plasma haptoglobulin concentration occurred non-specifically as a part of acute phase reaction during acute inflammation. This work confirmed inflammation superceding haemolysis in genitourinary schistosomiasis.

C - reactive protein (CRP) was observed to increase in the infected children when compared with control. This was as a result of inflammatory response to schistosome antigens. Although, it was observed that, CRP reduced insignificantly in the lightly *S. haematobium* infected children as compared with the heavily infected. The explanation for this could be as a result of more complement activation by CRP in heavily infected.

This CRP, is known to initiate alternate pathway of complement activation (Stites 1980) and expected to be significantly higher in heavily infected than lightly infected as an inflammatory response to higher number of eggs laid by schistosome adults. Nonetheless most of the CRP produced were observed to have been consumed at a faster rate in heavily infected by alternate pathway of complement activation.

Both α-2-macroglobulin and haptoglobulin were observed to increase significantly amongst the infected children when compared with schistosomiasis free controls. This can be explained in line with inflammatory response to schistosoma antigens.

Stites (1980) opined that caeruloplasmin could function as an oxygen-radical scavenger in inflammatory circumstances and therefore caeruloplasmin increase, during genitourinary schistosomiasis was obvious, given the possible increase in immunological reactions by immune competent cells against schistosome antigens.

Table 4
Purified protein derivation (PPD - Mantoux skin Test) on control, and *S. haematobium* infected children (treated and untreated)

Subjects	No. examined	Mean diameter (mm) of Mantoux skin test in treated children	Mean diameter (mm) of Mantoux skin test in untreated children
Control	50	4.8 ± 2.5	4.0 ± 2.0
Lightly infected (1-50 eggs/10ml urine)	75	5.2 ± 3.4	4.4 ± 3.3
Heavily infected (>50 eggs/10ml urine)	75	8.7 ± 5.6	7.6 ± 5.2
Control and lightly infected compared		> 0.02	> 0.01
Control and heavily infected compared		> 0.01	> 0.01
Lightly infected and heavily infected compared		< 0.02	< 0.04

Immune modulation of host immune systems against schistosome antigens, to permit adult schistosome worms existed and has been established by high serum concentration lymphokines being associated with acute intestinal schistosomiasis (Robert *et al* 1993) and a low level of lymphokines associated with chronic stage of intestinal schistosomiasis (El Missiry *et al* 1994).

To date, inhibitory substances to leucocyte migration in the serum of *S. haematobuim* infected patients have not been determined. This work has established that leucocyte migration inhibitory factors are present in the sera of *S.haematobium* children. This was because the sera from both lightly and heavily infected children significantly reduced the migration index of leucocyte when compared with the sera from the non-infected control. The result showed the presence of serum factor(s) particularly in heavily infected children prevent phagocytes from moving off the sites of infections. This augmented inflammation, phagocytosis and granuloma formation. It increased leucopenia from the peripheral blood counts. Acute phase proteins and other serum factors had contributed to this inhibition of leucocyte migration, since high concentration of C-reactive protein was suppressive in *in vitro* lymphocyte responses (Stites 1980). Incubation of sera from

S.haematobium infected pupils at temperature of 56˚C for 30 min did not significantly inhibit migration of leucocytes in the control and infected children. This showed that leucocytes migration substance is likely to be a heat labile protein. Inhibitory tendency was high amongst the heavily infected, since this category was exposed to constant re-infection and therefore immune complexes were excessively formed due to production of excess antibodies. The immune complexes with inherent activation were responsible for haemorrhage.

Negative skin response of genitourinary schistosomiasis in children to tuberculin antigen was markedly low. This was contrary to the opinions of Wilkin and Brown (1977) who showed a reduced positive response to tuberculin streptococcal antigen among *S. haematobium* infected Gambians. This study affirmed a reduced negative skin response in the infected children and was a factor of adequate functioning of T- lymphocyctes in these infected children. Skin reaction was observed to increase in largeness of diameter of induration amongst the heavily infected when compared with lightly infected children. This was as a result of high amount of lymphokines and immune complexes (leucocyte migration inhibitor) in the serum of the heavily infected.

The high amount of serum migration inhibition lymphokines and immune complexes retained more leucocytes (phagocytes) at the site of tuberculin purified protein derivative injection with the resultant increase in skin reaction diameter. Post treated schistosomiasis children showed more positive and increased diameter of induration to Mantoux skin test when compared to the untreated children. This was as a result of the second injection of tuberculin (PPD) acting as boost.

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