AFRICAN JOURNAL OF MEDICINE and medical sciences

Volume 32, No 3 September 2003

EDITOR
B. O. OSOTIMEHIN

ASSISTANT EDITOR
A. O. UWAIFO

ISSN 1116—4077

Afr. J Med. med. Sci. (2003) 32, 263-267

The effects of ageing on the immune response to *schistosoma haematobium* and hookworm by measuring circulating immune complexes, C3, Igg, Iga and Igm levels in residents of Omi Dam Area of Kogi State, Nigeria

GO Oyeyinka[a], IA Awogun[a], TM Akande[a], JA Awarun[a], OG Arinola[b] and LS Salimonu[b]
[a]Faculty of Health Sciences, University of Ilorin, P.M.B 1515, Ilorin,
[b]Chemical Pathology, College of Medicine, University College Hospital, Ibadan, Nigeria.

Summary
In this study, the effects of infestation (*with Schistosoma haematobium* or hookworm) during host ageing on the serum levels of circulating immune complexes (CIC), C3, IgG, IgA and IgM were examined in residents of Omi dam area of Kogi state, Nigeria. *S. haematobium*-infested and hookworm-infested individuals showed no significant alteration in the levels of CIC, C3, IgG, IgA and IgM in comparison with controls. These levels were the same in infested subjects and controls even when the patients were pooled. Infested old people had the same concentrations of serum CIC, C3 IgG and IgM in comparison with infested young people but IgA levels were higher in the aged group ($t=2.100$; $P<0.05$); and were significantly correlated with age ($r=0.301$; $P<0.05$). No significant increase in CIC levels with rising age ($r=0.123$; $P>0.20$) was observed in the overall-all population of infested subjects; and infestation in old age did not alter CIC, C3, IgG, IgA and IgM levels in comparison with uninfested young people. For the uninfested, IgG, IgA and IgM values were similar in the aged and the young but the levels of CIC were higher ($t=2.156$; $P<0.05$; $r=0.280$; $P<0.05$) and C3 lower ($t=3.313$; $P<0.01$; $r=-0.236$; $P>0.10$) in the aged. The results of this study suggest that the elevated CIC levels found in old people is age-related; and that the contribution of parasitic infestation to these raised levels is uncertain.

Keywords: *CIC, C3, Immunoglobulins, Schistosoma haematobium,Hookworm, Ageing.*

Resume
Les effects d'infection du schistosome hemetobum durant l age adulte sur les taux du serum des complexes immunitaire circullant dans le corps (C3, IgG, IgA & IgM) étaient examinés chez des residents d'Omidan dans la province de Kogi au Nigeria. Les individus du S hemotobum et des Vas n' avaient aucune altération significative du taux de C3, IgA, IgM comparés au groupe de controle. Ces taux étaient egaux aux 2 groupes. La différence d'age n'avait aucun effect sur les concentrations du serum du CIC, C3, IgM , Ig G , amis les taux du IgA étaient plus elevés chez les plus agés ($t=2.1$, $P<0.005$) et correlaient significativement avec l age ($r= 0.301$, $P<0.05$). Il n'y avait pas d 'augmentation significative du CIC avec l'agmentation ($r=0.123$, $P>0.20$) chez ces subjects infectés. La jeunesse non infectés avait des valeurs du IgA , IgG , IgM simmilaire a ceux des plus agés mais certains CIC étaient elevés ($t=2.156$' $P<0.005$; $r=0.280$ ' $P<0.05$) et une valeur faible de C3 ($t=3.313$; $P<0.001$, $r=0.216$'$P>0.10$) Ces résultats suggérent que l'élevation du taux du CIC cehz les plus agés est lié a l'age et la constitution des infection parasitologiques a cette agmentation reste incertain.

Correspondence: Dr. G.O. Oyeyinka, Department of Chemical Pathology and Immunology, Faculty of Health Sciences, University of Ilorin, P.M.B. 1515, Ilorin, Nigeria.

Introduction

Immune responsiveness declines with increasing age [1]. For instance, those who show an absence of delayed cutaneous hypersensitivity have been observed to have a much higher risk of impending death compared to those with a positive skin response [2]. The decline in immune response with age is not an invariable occurrence as almost one-third of the healthy elderly have immunological functions at levels seen in younger age groups [3]. Many parasitic infestations are also known to be accompanied by immunodepression [4]. There is an increase in the levels of CIC in the healthy elderly compared to young controls [5,6]. This may be due partly to the vulnerability of old people to infestation. Immune complexes suppress immune responses [7,8]. CIC induced immunodepression is one way parasitic infestations are thought to produce lowered immune responsiveness. Indeed, acute schistosomiasis involves immune complex formation [9] and lesions due to complexes of specific anti-parasite antibodies and parasite antigens occur in a variety of parasitic diseases [10]. This study examines the effect of ageing on the immune response to *Schistosoma haematobium* and hookworm by measuring CIC, C3 and immunoglobulins G, A and M levels in residents of Omi dam area of Kogi State, Nigeria.

Subjects and methods

Background of subjects and sample collection

Samples for this study were obtained from residents of the villages of Omi and Ogga, both located near Omi dam in Yagba West Local Government Area of Kogi State, Nigeria. Omi village is by Omi dam and Ogga is an outlay about two kilometers east of the dam. The two villages, with a population of 7500 and 10,000 respectively, were chosen because of their closeness to the dam. The people of these rural communities are peasant farmers and petty traders. Most of the adult population at home did not receive formal education while the children of school age attend primary schools located in the villages. In all, 29 people infested with *S. haematobium* (mean age: 18.7±19.7; range: 7–80 years old), and 10 with hookworm (mean age: 60.3±26.8; range: 11-90 years old) were studied. Thirty-four apparently healthy individuals (mean age: 47.7±28.7; range: 5-80 years old) from the two communities, who were long term residents, served as controls. Thirteen other hookworm infested people (aged 59.2±26.5; range: 23-100 years) from the two villages who had only CIC measurements but no C3 or immunoglobulin estimation, were included in the study. These samples

were obtained from a total of 153 villagers studied (age range: 5-100 years). The samples that were excluded from analysis were from those infested with other parasites. Those who had no ova or parasite in their stool and urine, and were otherwise healthy, were 58. Thirty-four of these had the five parameters studied done on them while only CIC was measured in the remaining 24 samples. Six individuals were doubly infested with both parasite. Informed consent was obtained from the community leaders before embarking on the project.

Blood sample (3.0ml) collected from each subject (between 8.00 and 13.00 GMT) was separated by centrifugation after clotting and retraction, and the serum stored at –20°C until analysed. Urine (about 15ml) and stool samples collected in the morning hours before leaving for farm or market (usually between 6.00 and 7.00 GMT) were also supplied by each participant and preserved with 10% formaldehyde until they were examined. Those infested were proved positive for *S. haematobium* eggs in urine or for hookworm ova in stool; and had no other severe or chronic disorders. About 10ml of urine was centrifuged at 300 xG for 5 minutes and a drop of the deposit examined under coverslip on a microscope slide with x10 and x40 objectives. A small portion of stool sample was first emulsified in 1 drop of saline and if necessary also in 1 drop of 1% Lugol's iodine on a microscope slide and then examined under a coverslip with x10 and x40 objectives. Differential leucocyte counts were performed on the subjects who were also screened for malaria parasitaemia and filariasis (*Wuchereria bancrofti)* on the same Leishman-stained thin blood film.

Laboratory investigations

CIC were measured by the polyethylene glycol (PEG) precipitation method (11). Serum samples stored at –20°C and thawed only once were diluted 1:3 with borate buffer (pH 8.4). 0.22ml of each diluted serum was added to 2.0ml of 4.166% PEG 6000 solution and mixed thoroughly. A blank was set up for each diluted serum by mixing 2.0ml of borate buffer with 0.22ml of the diluted serum. Incubation was for one hour at 30°C. The optical densities were read at 450nm in a spectrophotometer against blank. The concentration of CIC in each sample was read off a standard immune complex calibration curve prepared as described by Haskova *et al.* [11].

Serum C3 and immunoglobulins G, A and M levels were estimated by the single radial immunodiffusion technique [12] using commercial monospecific antisera (Serotec, Oxford, England) to human C3c, IgG, IgA and IgM respectively. The levels of the immunoglobulins were measured against serum standards (The Binding Site, Birmingham, England) obtained from the laboratory of Dr. D. Catty of the Department of Infection, The Medical School, University of Birmingham, Birmingham, United Kingdom. C3 levels were measured against a pooled serum standard obtained from nine adult blood donors in Ilorin, Nigeria.

Subjects >35 but <65 years of age were excluded in the age-related comparisons in Tables 5-7 because they were too few in number for any meaningful statistical analysis.

Data analysis

Significance of differences between mean values was assessed by students' t-test. P-values less than 0.05 were regarded as significant. Effects of ageing on CIC, C3, IgG, IgA and IgM levels in infestation and in health were examined by determining the coefficient of correlation (r) for each of these parameters with age.

Results

Leishman-stained thin blood films, after extensive search, revealed that 50% of all schistosome and/or hookworm infested individuals studied and 51.6% of control individuals had asymptomatic scanty malaria parasitaemia. This asymptomatic condition was found in 40.9% of those infested with *S. haematobium and* 75% of those infested with hookworm. No filarial worms were found in Leishman-stained thin blood films in any of the subjects studied including infested and control individuals. The mean concentrations of CIC, C3 and immunoglobulins G, A and M obtained in *S. haematobium* infested and uninfested individuals residing near Omi dam are displayed in Table 1.

Table 1: Mean (±1s.d) serum concentrations of CIC, C3, IgG, IgA and IgM in S. *haematobium* infested and uninfested individuals (≥35 years old) residing near Omi dam

Subjects	CIC(g/l)	C3 % pooled std	IgG(g/l)	IgA(g/l)	IgM(g/l)
Infested	0.34±0.27	112.3±23.1	26.26±6.49	2.51±1.10	1.32±1.24
Subjects	n=25	n=24	n=20	n=24	n=24
Uninfested	0.30±018	1118±18.0	24.91±5.93	3.10±1.71	0.90±0.52
Subjects	n=13	n=13	n=7	n=13	n=13
t	0.544	0.073	0.506	1.124	1.442
P	>0.20	>0.20	>0.20	>0.20	>0.10

The mean (±1s.d) age of infested subjects was 11.3±3.0 years and for uninfested subjects: 13.5±7.3 years; with no significant age difference between the two groups (t= 1.072; P> 0.20).

There were no significant alterations in the levels of these components due to infestation with *S. haematobium*. The mean concentrations of the components obtained in hookworm infested and uninfested individuals residing in the study area are shown in Table 2 with no differences between them.

Table 2: Mean(±1s.d) serum concentrations of CIC, C3, IgG, IgA and IgM in hookworm infested and uninfested individuals (≥65 yrs old) residing near Omi dam.

Subjects	CIC(g/l)	C3 % pooled std	IgG(g/l)	IgA(g/l)	IgM(g/l)
Infested	0.39±0.36	100.7±24.4	26.73±5.98	4.14±1.89	1.71±1.36
Subjects	n=15	n=7	n=5	n=7	n=7
Uninfested	0.45±0.20	87.3±22.5	24.84±8.43	4.28±1.75	1.23±0.58
Subjects	n=17	n=17	n=14	n=17	n=15
t	0.572	1.250	0.540	0.168	1.990
P	>0.20	>0.20	>0.20	>0.20	>0.20

The mean (±1s.d) age of infested subjects was 75.9±11.4years and for uninfested subjects: 72.1±7.5 years; with no significant age difference between the two groups (t= 1.098; P> 0.20).

There was accident with one of the samples for CIC determination. Table 3 compares the mean values of the five parameters between infested and uninfested aged subjects and found no significant differences. Similar results were obtained between infested and uninfested young subjects (Table 4).

Table 3: Comparison of mean (±1s.d) serum concentration of CIC,C3,IgG,IgA and IgM between infested (with *S.haematobium* and/or hookworm) and uninfested aged individual (≥65 years old) residing near Omi dam

Subjects	CIC(g/l)	C3	IgG(g/l)	IgA(g/l)	IgM(g/l)
		% pooled std			
Infested	0 46±0 42	101.3±25.6	27.37±5.34	3.69±1.77	1.35±1.15
Subjects	n=20	n=12	n=7	n=12	n=12
Uninfested	0.45±0.20	87.3±22.5	24.84±8.43	4.28±1.76	1.23±0.58
Subjects	n=17	n=17	n=14	n=17	n=15
t	0.095	1.524	0.836	0.886	1.330
P	>0.20	>0.10	>0.20	>0.20	>0.20

The two aged subject groups were of similar mean ages (t= 1.138;P> 0.20.

Table 4: Comparison of mean (±1s.d) serum concentration of CIC, C3, IgG, IgA andIgM between infested (with *S.haematobium* and/or hookworm) and uninfested young individuals (≤ 35 years old) residing near Omi dam

Subjects	CIC(g/l)	C3	IgG(g/l)	IgA(g/l)	IgM(g/l)
		% pooled std			
Infested	0.36±0.26	111.4±22.3	25.37±7.74	2.54±1.08	1.32±1.18
Subjects	n=35	n=30	n=25	n=30	n=30
Uninfested	0.30±0.18	111.8±18.0	24.91±5.93	3.10±1.71	0.90±0.52
Subjects	n=13	n=13	n=7	n=13	n=13
t	0.902	0.062	0.169	1.090	1.625
P	>0.20	>0.10	>0.20	>0.20	>0.10

The two young subject groups were of similar mean ages (t= 0.040; P> 0.20).

Table 5: Mean (±1s.d) serum concentrations of CIC, C3, IgG, IgA and IgM in *S. haematobium* and/or hookworm infested ages and young individuals residing near Omi dam

Subjects	CIC(g/l)	C3	IgG(g/l)	IgA(g/l)	IgM(g/l)
		% pooled std			
Infested aged (74.1±7.5 range: 65-90 years	0.46±0.42 n=20	101.3±25.6 n=12	27.37±5.34 n=7	3.69±1.77 n=12	1.35±1.15 n=12
Infested young (11.8±3.2 range: 5-35 years)	0.36±0.26 n=35	111.4±22.3 n=30	25.37±7.74 n=25	2.54±1.08 n=30	1.32±1.18 n=30
t	0.964	1.197	0.786	2.100	0.076
P	>0.20	>0.20	>0.20	>0.05	>0.20

Subjects > 35 but <65 years of age were too few (2) for meaningful statistical analysis, and were therefore excluded. correlation of the parameters with age, using the total sample (n= 57) of infested individuals including those aged >35 but <65 years, gave the following:- CIC. r= 0,123; P>0.20; C3: r=0,270; P>0.05; IgG: r=0, 110; P>0.20; IgA: r=0.301; P>0.05; IgM: r=0,101; P>0.20

Age-related effects of infestation with S. *haematobium* and/or hookworm on CIC, C3, IgG, IgA and IgM are shown in Table 5. Mean serum IgA levels were higher in infested aged people in comparison with infested young subjects. Other parameters had similar mean values between the two groups. Correlation analysis showed that the rise in CIC levels with increasing age was not significant (r=0.123; *P*>0.20) in the overall population (n=57) of infested subjects analysed. Except for IgA, there was also no significant correlation with age in the levels of the other parameters in infested individuals – C3: r=-0.270; *P*>0.05; IgG: r=0.110; *P*>0.20; IgA: r=0.301; *P*<0.05; IgM: r=0.101; *P*>0.20. The levels of CIC, C3, IgG, IgA and IgM were not altered by the combined effects of old age and infestation (Table 6). Table 7 presents age-related changes in the values of CIC, C3, IgG, IgA and IgM in apparently healthy uninfested people in the study area.

Table 6: Mean (±1s.d) serum concentrations of CIC, C3, IgG, and IgM in S. haematobium and/or hookworm infested aged and uninfested young individuals.

Subjects	CIC(g/l)	C3	IgG(g/l)	IgA(g/l)	IgM(g/l)
		% pooled std			
Infested aged (74.1±7.5 range: 65-90 years	0.46±0.42 n=20	101.3±25.6 n=12	27.37±5.34 n=7	3.69±1.77 n=12	1.35±1.15 n=12
Uninfested young (13.5±7.3 range: 5-35 years)	0.36±0.18 n=13	111.8±18.0 n=13	24.91±5.93 n=7	3.10±1.71 n=13	0.90±0.52 n=13
t	1.509	1.177	0.816	0.846	1.244
P	>0.10	>0.20	>0.20	>0.20	>0.20

Subject >35 but <years of age were too few for meaningful statistical analysis, and were therefore excluded.

Table7: Mean (±1s.d) serum concentrations of CIC, C3, IgG, and IgM in uninfested aged and young individuals residing near Omi dam..

Subjects	CIC(g/l)	C3	IgG(g/l)	IgA(g/l)	IgM(g/l)
		% pooled std			
Infested aged (72.1±7.5 range: 65-90 years)	0.45±0.20 n=17	87.3±22.5 n=17	24.84±8.43 n=14	4.28±1.76 n=17	1235±0.58 n=15
Uninfested young (13.5±7.3 range: 5-35 years)	0.36±0.18 n=13	111.8±18.0 n=13	24.91±5.93 n=7	3.10±1.71 n=13	0.90±0.52 n=13
t	2.156	3.313	0.022	1.850	1.588
P	>0.05	>0.01	>0.20	>0.05	>0.10

Subject >35 but <65 years of age were too few for meaningful statistical analysis, and were therefore excluded. Correlation of the parameters with age, using the total sample of uninfested individual including those aged >35 but 65 years, gave the following: CIC: r=0,280; P<0.05;C3: r=0.236; P>0.10; IgG: r=0.060; P>0.20; IgA: r=0.168; P>0.20; IgM: r=0.273; P>0.10.

Similar mean values were obtained for the immunoglobulins but CIC levels were higher (t=2.156; *P*<0.05) and C3 lower (t=3.313; *P*<0.01) in the apparently healthy aged. CIC levels showed significant correlation (r=0.280, n=58, *P*<0.05) with age. Correlation of age with CIC excluding the 24 healthy indi-

viduals who had only CIC estimation gave r=0.312; $P>0.05$. The other parameters studied showed no significant correlation with age in healthy people – C3: r=-0.236; $P>0.10$; IgG: r=0.060; $P>0.20$; IgA: r=0.168; $P>0.20$; IgM: r=0.273; $P>0.10$.

Discussion

Consequent on the immunodepression of infection [4,13-15] and old age [3] it can be expected that the levels of circulating immune complexes and other immunologic indices are altered during infection and ageing. In this study, in an endemic environment, infestation with *S. haematobium or* hookworm did not affect serum CIC, C3 and immunoglobulins G, A and M concentrations in comparison with residents found uninfested at the time of sample collection. These results do not rule out the possibility of differences in the types of immune complexes (IgG or IgM) or in the relative proportion of IgG isotypes. The result is surprising, especially given the production of circulating antigens from schistosomes [16]. However, T cell hyporesponsiveness with both Th1 and Th2 cytokines down-regulated was found in active schistosome re-infestation in comparison with controls who had recovered from infestation with the parasite two years before [15]. This may lead to the production of only small amounts of antibody.

Although acute schistosomiasis involves immune complex formation [9], this study could not demonstrate any significant increase in the levels of CIC, as a result of these infestations, over endemic controls. The complexes responsible for the lesions of parasitic infestations [10] may be bigger complexes than the ones that circulate [4], and may thus be subject to phagocytic clearance. The unaltered C3 levels observed suggest that immune complex-associated complement consumption is unlikely in these infestations. A lack of significant alteration in all three classes of immunoglobulins studied differs from previous reports which indicated elevated IgA and IgG [17] and reduced IgM [18] levels in schistosomiasis. The reason why the immunoglobulin levels remained unaltered in infestation with *S. haematobium* or hookworm in the present study is not obvious but it does not exclude the possibility of changes in the levels of parasite-specific antibodies. Splitting infested and uninfested groups on the basis of one urine or stool sample as was done in this study may have only revealed infestation-free spell in the controls; and an assumption that the whole population was infested would explain the lack of differences between groups in the parameters studied.

Infested old people had similar CIC levels to those found in infested young individuals, and the values did not show significant correlation with age in these infested subjects. However, uninfested aged subjects had significantly higher mean CIC levels compared to the uninfested young, and these levels correlated significantly with age. This result agrees with the general belief that CIC levels are increased during ageing [5,6]. The failure to find significant difference in CIC levels between infested old and infested young people or the uninfested young controls suggests that age per se is responsible for elevated levels found in old people. It was believed that elevated immune complex concentrations in sera from old subjects could result from the vulnerability of old people to infestation or from the age-associated increase in the prevalence of auto antibodies [6]. The observations made above suggest that age may be a principal contributor to elevated CIC levels in old age; and that the role of parasitic infestation in this condition is uncertain.

Significantly higher mean serum IgA level obtained in the infested aged compared to infested young control group may be age-related as IgA tends to increase with age [19]. Indeed, IgA showed significant correlation with age in infested subjects in this study. However, similar serum IgA concentrations were obtained in healthy aged and uninfested young individuals; and IgA did not correlate with age in healthy subjects. It is not clear why C3 levels were significantly reduced in healthy aged compared to healthy young controls. It is not likely that complement activation was responsible for the reduction as apparently healthy individuals were involved. It is possible that there is diminished C3 production with age; especially as C3 showed negative correlation with age in infested as well as uninfested subjects in this study, but these were not statistically significant.

Acknowledgements

We thank the University of Ilorin, Ilorin, Nigeria for Senate Research Grant (1995) to Professor Awogun, Dr. Oyeyinka and Dr. Akande from which this study benefited. We are grateful to His Royal Highness, Oba Ayodele Ogun, the Olomi of Omi; and His Royal Highness, Oba D.A. Kumolu, the Ologga of Ogga for permission to carry out this study. Population figures were obtained from West Yagba Local Government Authority Office, Odo Ere.

References

1. Murasko DM, Weiner P and Kaye D. Decline in mitogen induced proliferation of lymphocytes with increasing age. Clin. Exp. Immunol. 1987;70:440-448.
2. Roberts-Thompson IC, Whittingham S, Youngchaiyud U and Mackay IR. Ageing, immune response and mortality. Lancet 1974;ii:368-370.
3. Chandra RK Influence of nutrition on immunocompetence in the elderly. in: Cunningham-Rundles S (ed): Nutrient modulation of the immune response. New York, Marcel Dekker, 1993,455-465.
4. Greenwood BM and Whittle HC. Immunology of Medicine in the tropics. London, Edward Arnold, 1981;21-61.
5. Goodwin JS, Searles RP and Tung SK. Immunological responses of a healthy elderly population. Clin. Exp. Immunol. 1982;48:403-410.
6. Oyeyinka GO, Salimonu LS and Ogunsile MO. The role of circulating immune complexes; antinuclear and rheumatoid factor autoantibodies in aging in Nigerians. Mech. Ageing Dev. 1995;85:73-81.
7. Baldwin RW and Robins RA. Factors interfering with immunological rejection of tumours. Br. Med. Bull. 1976;32:118-123.
8. Morgan EL and Tempelis CH. The requirement for the Fc portion of antibody in antigen-antibody complex-mediated suppression. J. Immunol. 1978;120:1669-1671.
9. Colley DG. Schistosomiasis. in: Mahmoud AAF, (ed): Clinical tropical medicine and communicable diseases; London, Balliere Tindall, 1987,315-332.
10. Scott PA and Sher A. Immunoparasitology. in: Paul WE (ed): Fundamental Immunology. Philadelphia:, Lippincott-Raven, 1993,1179-1210.
11. Haskova V, Kaslik J, Riha I, Matl I and Rovensky J. Simple method of circulating immune complex detection in human sera by polyethylene glycol precipitation. Z. Immunitaets, Forsch. Immunobiol. 1978;154:399-406.

12 Salimonu LS, Ladipo OA, Adeniran SO and Osunkoya BO. Serum immunoglobulin levels in normal, premature and postmature newborns and the mothers. Int. J. Gynaecol. Obstet. 1978;16:119-123.

13. Olatunde BO and Onyemelukwe GC. Immunosuppression in Nigerians with hookworm infestation. Afr. J. Med. Med. Sci. 1994;23:221-225.

14. Viana IRC, Sher A, Carvalho OS, Massara CL, Eloi-Santos, SM, Pearce EJ. et al. Interferon-gamma production by peripheral blood mononuclear cells from residents of an area endemic for *Schistosoma mansoni*. Trans. Roy. Soc. Trop. Med. Hyg. 1994;88:466-470.

15. Grogan JL, Kremsner PG, Deelder AM and Yazdanbakhsh M. Antigen-specific proliferation and interferon-gamma and interleukin-5 production are down-regulated during *Schistosoma haematobium* infestation. J. Infectious Dis. 1998;177:1433-1437.

16. Naus CW, Van Dam GJ, Kremsner PG, Krijger FW and Deelder AM. Human IgE, IgG subclass, and IgM responses to worm and egg antigens in *Schistosomiasis haematobium* : a 12-month study of reinfestation in Cameroonian children. Clin. Infect. Dis. 1998;26:1142-1147.

17. Arinola OG and Salimonu LS. Serum immunoglobulins in Nigerians with urinary schistosomiasis. E. Afr. Med. J. 1997;74:331-334.

18. Dessaint JP, Capon M, Bout D and Capron A. Quantitative determination of specific IgE antibody to schistosome antigens and serum IgE levels in patients with *S. mansoni* or *S. haematobium*. Clin. Exp. Immunol. 1975;20:427-436.

19. Oyeyinka GO, Salimonu LS, Williams AIO, Johnson AOK, Ladipo OA and Osunkoya BO. Range of normal serum immunoglobulin (IgG, IgA and IgM) values in Nigerians. Afr. J. Med. Med. Sci. 1984;13:169-176.