Serological Studies on Nephrotic Syndrome of Quartan Malaria in Uganda

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Summary : Ugandans with high malarial antibody titres have been found also to have higher IgM levels. Patients with active nephrotic syndrome have higher IgM and malarial antibody levels than both controls and nephrotics in remission, an extreme increase in these factors being found in patients in whom immune complexes were present.

Introduction

Hendrickse and Gilles (1963) reported on the association between the nephrotic syndrome and quartan malaria in West African children. Evidence has since accumulated which suggests that circulating antigen-antibody complexes are involved in the renal damage (Soothill and Hendrickse, 1967; Allison *et al.*, 1969; Ward and Kibukamusoke, 1969). We thought that this apparently abnormal immunological response to malarial infection might be reflected in the serum immunoglobulin and malarial antibody levels, and the present study represents an attempt to assess the immunoglobulins IgM and IgG and the malarial antibody levels in a group of nephrotics in Uganda.

Materials and Methods

The study group consisted of 53 Ugandans with the nephrotic syndrome (34 active and 19 in remission) and a comparable group of 19 non-nephrotic controls. Malarial antibody was measured by the indirect fluorescent antibody method described by Voller (1964). The antigen used was thin blood smears of *Plasmodium falciparum* made from experimentally infected *Aotus trivirgatus* (owl monkeys). The values given are the reciprocal of the terminal serum dilutions yielding detectable fluorescence.

The estimations of immunoglobulins IgG and IgM were carried out by the radial diffusion method with specific antibody in agar-gel plates (Mancini *et al.*, 1963) purchased from Hyland Laboratories, California, U.S.A. Sera were diluted for re-testing when the diameter of the precipitation zones were greater than that of the highest standard immunoglobulin preparations available. (IgG and IgM values are expressed as mg./100 ml. serum).

The Student *t* test was used for the statistical analysis of the results.

Results

The individual and mean values for IgG, IgM, and malarial antibody titres in active nephrotics, nephrotics in remission, and the control Ugandans are given in Table I. The IgG

TABLE I.—*Immunoglobulin and Malarial Antibody Levels*

	Immunoglobulin Levels (mg./100 ml.)		Malarial Antibody Levels (Reciprocal of Fluorescent Antibody Titre)
	IgG	IgM	
Ugandans with Active Nephrotic Syndrome			
1	684	140	80
2	5,040	440	10,240
3	860	224	20
4	1,372	660	160
5	3,040	700	5,120
6	2,500	270	160
7	1,240	800	5,120
8	1,880	460	640
9	1,992	230	<20
10	1,608	234	5,120
11	576	270	5,120
12	3,040	81	40
13	608	152	320
14	2,040	185	5,120
15	1,660	545	10,240
16	1,900	295	1,280
17	1,900	130	5,120
18	330	165	<20
19	260	270	20
20	1,112	245	80
21	500	185	1,280
22	1,200	105	<20
23	2,840	335	10,240
24	1,280	270	<20
25	1,770	545	10,240
26	2,320	130	80
27	810	1,440	640
28	1,020	1,118	10,240
29	1,180	430	5,120
30	1,360	460	2,560
31	1,400	185	20
32	1,800	1,800	5,120
33	1,020	185	640
34	1,300	2,206	10,240
Mean (standard error)	1,572 ± 163	467 ± 84	3,248 ± 662
Ugandans whose nephrotic syndrome was in state of remission			
1	3,200	220	5,120
2	1,880	107	80
3	2,500	1,000	5,120
4	1,700	198	10,240
5	860	66	20
6	1,880	160	2,560
7	760	276	20
8	1,608	236	5,120
9	3,040	236	20
10	3,040	246	5,120
11	1,992	205	10,240
12	440	147	<20
13	1,000	185	4,120
14	1,180	92	80
15	1,992	245	5,120
16	1,560	117	<20
17	2,040	165	80
18	1,800	205	80
19	1,372	105	40
Mean (standard error)	1,781 ± 178	221 ± 45	2,852 ± 801
Ugandans without nephrotic syndrome			
1	3,200	246	20
2	2,600	360	640
3	1,500	220	80
4	2,600	220	80
5	2,200	236	<20
6	1,800	238	<20
7	1,520	98	80
8	2,500	220	160
9	2,320	236	40
10	2,200	224	1,280
11	1,800	128	80
12	Not done	224	640
13	1,992	220	160
14	2,880	238	2,560
15	1,880	220	<20
16	2,600	260	160
17	2,100	242	40
18	3,040	254	160
19	4,400	300	640
Mean (standard error)	2,392 ± 165	230 ± 12	360 ± 144

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values of the three groups are not significantly different. The group with active nephrotic syndrome have significantly higher IgM levels than the controls and the nephrotics in remission ($0.05>P>0.025$). Malarial fluorescent antibody levels are higher in the active nephrotics than in the control group ($0.01>P>0.005$). The nephrotics in remission also have higher mean malarial antibody levels than the controls, but the difference is not statistically significant ($0.10>P>0.05$).

In Table II the immunoglobulin levels are analysed with

TABLE II.—*Immunoglobulin Levels of Ugandans Analysed in Relation to Their Malarial Antibody Titres*

	Low Malarial Antibody Levels (F.A. Titre 320 or less)			High Malarial Antibody Levels (F.A. Titre above 320)		
	No.	IgM (mg./100 ml.) Mean (Standard Error)	IgG (mg./100 ml.) Mean (Standard Error)	No.	IgM (mg./100 ml.) Mean (Standard Error)	IgG (mg./100 ml.) Mean (Standard Error)
Active nephrotics ..	14	223 ± 38	1,354 ± 223	20	638 ± 128	1,763 ± 220
Nephrotics in remission	10	151 ± 22	1,486 ± 266	9	299 ± 88	2,101 ± 234
Controls	14	217 ± 12	2,212 ± 141	5	269 ± 27	3,020 ± 480 (based on 4 values only)
All groups combined	38	202 ± 16	1,709 ± 129	34	494 ± 83	1,984 ± 174

respect to malarial antibody levels. The data are analysed in this way because a high titre of malarial antibody indicates that an individual has experienced malaria recently. The IgG values are similar in the high and low malarial antibody groups. The mean IgM value, however, is much greater in the high malarial antibody group ($0.005>P>0.0025$). The active nephrotics with high malarial antibody values have even higher IgM concentrations in their sera than both the remitted nephrotics and controls with high malarial antibody titres ($0.05>P>0.025$).

The data of Ward and Kibukamusoke (1969) on the presence or absence of immune-complex deposition in renal biopsies is utilized for further analysis of our results on serum immunoglobulin levels and malarial antibody levels. Twenty-two of their nephrotic patients are included in the present study and the results are given in Table III. The IgM is sig-

TABLE III.—*Immunoglobulin and Malarial Antibody Levels in Nephrotic Ugandans Analysed in Relation to Presence or Absence of Immunoglobulin Deposition in Renal Biopsies*

Fluorescent Antibody staining for Immunoglobulin deposits in renal biopsies	No.	Immunoglobulin levels (mg./100 ml.) Mean (Standard Error) IgG	IgM	Malarial Antibody Levels (Reciprocal of F.A. Titres) Mean (Standard Error)
Positive	7	1,986 ± 357	523 ± 119	3,763 ± 1,424
Negative	15	1,893 ± 318	241 ± 38	2,587 ± 822

nificantly higher ($0.01>P>0.005$) in the group with renal immune-complex deposits than in the group without such deposits. IgG and malarial antibody levels were similar in both groups.

Discussion

The present work shows that there is a strong correlation between the rise in IgM and high malarial antibody levels in both control and nephrotic groups of Ugandans. This suggests a common aetiological agent which is, in all probability, malaria. Unfortunately the serological techniques used here for the estimation of antibodies do not permit reliable identification of the species of malaria parasite which stimulated the antibody production.

The group of patients with the nephrotic syndrome had higher IgM and malarial antibody levels than the controls, but the IgG values were similar in both groups. Since *Pl. malariae* has been shown to be the causative agent of the African nephrotic syndrome (Hendrickse and Gilles, 1963; Kibukamusoke *et al.*, 1967) it is probably responsible for the higher serum concentrations of IgM and malarial antibody in the nephrotics. A possible explanation is that the malarial antibody is in the IgM fraction of the serum. This has, however, been excluded, as immunofluorescent tests for malarial antibody with monospecific labelled antisera to IgM and IgG show that most of the malarial antibody detected by the standard fluorescent antibody procedure is in the IgG class (Voller, unpublished).

Recent work (Allison *et al.*, 1969; Ward and Kibukamusoke, 1969) has shown that the nephrotics often have glomerular deposits of IgM, IgG, IgA, and complement, with IgM predominating. In this context it is particularly interesting that the high antibody group of active nephrotics should have significantly greater serum IgM concentrations than the high-antibody representatives in the controls and nephrotics in remission. Similarly the nephrotics with immune-complex glomerular deposits have higher IgM levels than nephrotics without the demonstrable immune complexes.

The nephrotic patients may represent individuals who produce an excessive amount of IgM in response to malarial infection, though it is conceivable that there is an abnormal host response that affects IgM and malarial antibodies independently.

Newer techniques for the precise identification of soluble malarial antigens and antibodies (Wilson *et al.*, 1969) may lead to greater resolution of the mechanism by which the nephrotic syndrome develops.

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