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# Secretory IgA and complement levels in patients with hookworm infection in Zaria

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#### Summary

As a continuing investigation into the immunological reactions involved in hookworm infection the levels of secretory IgA (sIgA): IgM, IgA, complement C3 and C4 were studied in 57 Nigerians with hookworm infection and compared with those of 28 healthy, normal controls. The hookworm patients were divided into three groups based on the degree of anaemia (Hb1-7 and hypochromia +++). Group II had moderate anaemia (Hb8-11, hypochromia ++ and Group III had no signs of anaemia despite the underlying hookworm infection. The patients also comprised those in whom hookworm was the sole infection and those with hookworm associated with other parasites. Significant differences in the results between each patient subgroup and the controls were analysed using the student t-test. IgA was significantly elevated in patients with anaemia of mild to moderate severity and in patients with hookworm only (P < 0.05) while sIgA was significantly elevated in all subgroups compared to controls (P < 0.05). IgM was significantly elevated in-patients with marked anaemia, inpatients without anaemia and in those with hookworm infection associated with other parasites (P < 0.05). The difference in IgG levels between patients and controls was not significant (P 0.1). C4 was significantly elevated in patients with marked moderate anaemia and those with hookworm only (P < 0.05) while C3 levels were not significantly different in the subgroups compared with controls. These results suggest the possibility of polyclonal B-cell activation by Tindependent antigens such as the polysaccharide cuticular antigens of the hookworms and the stimulation of the classical pathway of the complement system.

Keywords: Secretory, compliment, hookworm, infection

#### Résumé

En continuant l'investigation des reactions immunologiques inpliques dans les infections aux verres intestinaux le taux des IgA, Secretoire (SigA), IgM, IgA, le complement C3 et C4 avaient etudies chez 517 Nigerian aifant des infections de vere intestinourx, et compares a coux de 28 control normaux et en bonne sante. Les patients aifant les vers intestinaux avaient ete divises en 3 groupes bases sur le degree d'anenice observe. Les divisions avaient ete prononce (Hb1-7 et l'hypo chronic +++,) Groupe II avait une anemie modere (Hb8-11, l'hypo chronic +++) et le groupe III n'avait aucun signe d'anemie en depit de l'infection par les verres intestnaux. Les patients faile de maniere suivanle: Groupe I avait une anenie comprenaient ceux qui etaient infectes sealement aux verres intestinaux et ceux qui avaient des infections parasitaires. Les difference significatives ous resultats chez les patients des

Correspondence: Prof. G.C. Onyemelukwe, Dept. of Medicine, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria. differents sour griupes etaient analyse par le stdent B totest. Le taux d Iga etait eleve chez les patients ayant une arenie legere on medere et chez les patients ayant uniquement des infections par les verres intestinaux (P < 0.05). Le taue d'IgA etait eleve dans tout les smo-groupe compare aux controles. (P < 0.05). Le taux d'IgM etaiteleve chez les patients ayant ure forte anemic et ceux quo avaient d'autres infections parasitaire asocies a l'infections par les verves intestinaux (P < 0.05). Les diffrences des taux d'IgG entre ceux des patients et ceux des controls n'elaivent pas significati (P < 0.1) Le taux de C4 etait significativeuent eleve ches les patients ayant ane anemic prononce ceux ayant une anemic modere et ceux ayant senlement des infections auc nerres intestinaux (P < 0.05). Le taux de C3 n;etaient pas significativement different dans

tpns les fous-grouped compares aux controles. Ces resultats suggerent une activation polyclonate des cellules B par des aestigenes de type T independent telsque les antigenes polysacchavides enticulaire des werres intestinaux et la stimulation de la voie classique du system des comptements ummunitaires.

#### Introduction

Functional protective immunity has not been demonstrated in humans with hookworm infection under natural or experimental conditions [1]. On the contrary, it has been shown that iron and protein deficiencies hinder the efficient expulsion of worms in rats= [2], such expulsion being said to be a combined effect of antibodies, T-lymphocytes and Blymphocytes [1]. The immune response to infection with hookworms has also received little study. Non-significantly raised values of immunoglobulins and complement have previously been reported in hookworm infection [3,4] These were attributed to an inflammatory response to numerous infections in the tropics. Lewis and co-workers; using the fluorescent antibody technique (FAT), were also able to detect antibodies to the surface antigens of hookworm larva, although all these studies required standardization and improvement in the specificity of technique.

In the present study, immunoglobulin and complement values were studied in hookworm patients before and four (4) months after treatment, to understand their possible role in the pathogenesis of hookworm infection in Nigerian patients.

#### Materials and methods

Fifty-seven patients were studied, 28 healthy persons (21 males and 7 females served as controls and were aged 16 to 45 years (mean  $28 \pm 7$  years) having no signs or symptoms of other disease and no ova of hookworm in their stools. The patients consisted of 44 male and 13 females subjects and were

aged 13 to 70 years (means 30 ? 14 years). They were diagnosed as suffering from hookworm infection based on the recovery of hookworm eggs in their stools by the hanging droplet method [5] and screened to exclude other diseases other than stool parasite by full clinical and laboratory checkouts. Most of the patients species of the hookworm was also determined by the test-tube filter paper culture method of Hsien [6]. The controls were from Zaria and its environs.

Serum samples were taken form the hookworm patients and controls and stored at  $-20^{\circ}$ C until the analysis of the levels of immunoglobulins A, C and M and complements C3 and C4 were determined by the radial immunodiffusion technique using heavy chain specific goat anti-human globulin sera by the method of Mancini Carbonara and Hereman [7]. A peripheral blood total white cell count and differential stain slide, as well as thin blood film were performed on each patient and control according to standard haematological procedures [8].

All results obtained were analysed statistically using the students t-test.

#### Results

## Parasitological findings

The patients were confirmed to be hookworm infected patients based on egg recovery from stool specimens. The intensity of infection as indicated by the average egg excretion in stool was 800 ova per gram of stool (range 100 - 2900 eggs per gram stool). However, four patients (7%) with superinfection ranging from 15,200 to 111,200 eggs per gram of stool were recorded.

From the test-tube filter paper culture results, Necator americanus was predominant occurring alone in 67% of the 57 patients= samples. Ancylostoma duodenale was always found together with N. americanus in 5.26% of the samples.

#### Peripheral blood cell counts

The thin blood films consistently showed hypochromic anaemia in 49% of the cases. The mean haemoglobin level was 8.3 g/dl (? 2.96 g/dl) while the mean packed cell volume (PCV) was 33 ? 11% Table 1). A significant relationship (P < 0.05) was found between level of haemoglobin and ova load. Anaemia was assessed in the study in terms of hypochromia and level of haemoglobin and the patients were divided into three groups comprising those with marked anaemia (Hb1-7 g/dl), moderate anaemia (Hb8-11 g/dl) and no anaemia (12 gm/dl). The white blood cell counts in the various groups of hookworms patients studied and in controls are indicated in Table 1. The mean of each group compared with that of controls was significantly different (P < 0.05). The differential lymphocyte counts were not significantly different compared to the controls (P = 0.1). A mild degree of eosinophilia (1-6%) was observed in 77% of the hookworm cases. Only in 16% of the patients was marked eosinophilia (6%) observed.

#### Immunoglobulin and complement profiles

Serum levels of immunoglobulin and complement in hookworm patients and controls are indicated in Table 2.

Secretory IgA levels were significantly higher in all categories of hookworm anaemia patients compared to the controls (P < 0.001). Serum IgA levels were also significantly higher in patients with moderate anaemia (P < 0.05). The mean values of serum IgG were not significantly affected, while mean serum IgM levels were demonstrably altered in patients with marked anaemia and those without.

While C3 levels were not significantly different from those of controls C4 levels were significantly elevated in all patients (P < 0.05).

#### Discussion

The increased total white blood cell counts have been due to an increase in B-cells. Though B-cells were not studied in this survey, the increase in IgA and IgM may reflect indirectly B cell activation both specifically and non-specifically in hookworm infection. This is supported by the significantly elevated levels of IgN, IgA and secretory IgA (P < 0.05) which were recorded in patients in the different subgroups of this In previous studies in ascariasis, trichiurasis and study. hookworm infections, high values of immunoglobulin, especially IgG and IgA were found in both patients and controls [3,4]. However in this study, both patients and controls had normal levels of IgG while IgM levels were elevated. The elevation in IgM in marked anaemic patients was attributed to an intense antigenic stimulus by the worms under conditions of depressed T-lymphocyte function which could predispose to prominent T cell independent polyclonal antibody responses. This explanation is supported by the fact that in our other study of hookworm infected patients, total Tcells were most depressed in patients with marked anaemia and superinfection [unpublished]

High level of serum IgA was observed in patients in this study especially those with moderate anaemia. Similar findings have been reported in studies in hookworm infection in India [3] and *Entamoeba* histolytica *amuebic* liver abscess patients in Northern Nigeria [11]. The elevated IgA could be recruited as an immunologic barrier against larvae migrating from skin to the portal venous circulation and could play a role in the activation of the alternate complement pathway. As IgA and particularly secretory IgA is the predominant immunoglobulin in the mucosal defence system, it may along with other immunoglobulins like IgM and IgG, couple parasite antigens forming immune complexes which may activate complement C4 component of the classical pathway which was found elevated in this study.

Secretory IgA levels have been found in serum and other body fluids in intestinal hookworm infections in man [4,12] and low levels of secretory IgA have been suggested to predispose patients to colonization by the hookworm [12]. Secretory IgA may also act indirectly on the parasite through helper and suppressor T-cells with isotype specific receptors for IgA in an IgA, dependent cellular cytotoxicity reaction [12]. In this study, the elevated secretory IgA levels are more likely to be involved in immunological defence against the hookworm parasites. Besides, a link between secretory IgA and the eosinophilic response in hookworm infections has been suggested [13] as secretory IgA is a more efficient agglutinator than IgG as it has four combining sites. As our hookworm patients were not deficient in secretory IgA, it may be suggested that even the elevated levels of sectory IgA observed are not sufficient to effect expulsion of the works

Table 1: Differential analysis of peripheral blood mononuclear cells in hookworm patient=s subgroups and controls.

Cell Type	I Marked Anaemia	II Moderate Anaemia	III No Anaemia	IV Patients with hookworm	V Hookworm And other parasites	Total Hookworm Patients	Controls	P-Value
WBC X10 <sup>9</sup> /L	$5.12 \pm 3.22$ n = 15	$4.95 \pm 2.31$ n = 15	$4.40 \pm 1.82$ n = 27	$4.9 \pm 1.5$ n = 41	$4.1 \pm 1.5$ n = 16	$4.72 \pm 2.3$ n = 57	$3.76 \pm 1.5$ n = 28	•P<0.01
(Range)	(19-123)	(2.7-9.3)	(1.4-8.3)	(1.4-8.0)	(1.4-8.0)	(1.4-12.3)	(1.2-8.3)	
Lymphocytes x10 <sup>*</sup> /L (Range)	$2.20 \pm 1.64$ n = 15 (0.79-7.25)	$2.23 \pm 1.31 \\ n = 15 \\ (1.07-6.51)$	2.09 ± .75 n = 27 0.15-3.33	$2.27 \pm 1.28$ n = 41 (0.15-7.25)	$1.84 \pm 0.66$ n = 16 (0.72-3.06)	2.16± 1.15 n = 57 (0.15-7.25)	$1.80 \pm 0.87$ n = 28 (0.57-4.01)	
1.73 ? 1.17 n = 27 (0.26-5.12	$2.08 \pm 1.25$ n = 15 (0.42-4.52)	$1.99 \pm 1.01$ n = 15 (0.7-4.64)		1.91± 1.18 n = 41 (026-4.65)	$1.84 \pm 1.05$ n = 16 (0.53-5.12)	1.88± 1.14 n = 57 (0.26-5.12)	$1.77 \pm 0.75$ n = 28 (0.51-3.98)	
Eosinophils x 10 <sup>9</sup> /L	$0.25 \pm 0.14$	0.43± 0.30	0.38 ± 0.35	0.35 ± 0.31	0.40 ± 0.29	0.36± 0.30	0.057 ± 0.035	
(Range)	n = 15 (0.05 - 0.40)	n = 15 0.03-1.08	n = 27 (0.05-1.32)	n = 41 (0.05-1.32)	n = 16 (0.08-1.08)	n = 57 (0.03-1.32)	n = 28 (0.001-0.18)	•p<0.01
Monocytes x 10°/L	3.0 ± 1.35•	$1.5 \pm 0.85$	$1.9 \pm 0.51$	$2.2 \pm 0.6$	$1.7 \pm 0.54$	$2.0 \pm 0.58$	$1.5 \pm 0.6$	
(Range)	n = 15 (0.01-6.0)	n = 15 (0.01-3.2)	n = 27 (1.0-2.9)	n = 41 (1.0-3.5)	n = 16 (1.0-3.5)	n = 57 (0.01-6.0)	n = 28 (0.01-3.2)	

N. S. = refers to not statistically significant

= statistically significant difference between individual groups and controls

n = no of samples

Table 2:	Levels of serum immunor	globulins and complement in hookworm patient subgroups and contro	ols.
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Immunoglobulin /Complement class	I Marked Anaemia (15)	II Moderate anaemia (15)	III No Anaemia (27)	Iv Total hookworm patients (57)	Controls (28)	P. value (of each subgroup as compared with control)
SigA (mg/ml)	$8.9 \pm 7.1$	$17.2 \pm 15.11$	$10.2 \pm 8.06$	$11.7 \pm 10.5$	$2.79 \pm 2.07$	P < 0.001
IgA (i. U / ml)	(0.73 - 26.0) 156 ± 112	(1.77 - 41.6) 244 ± 212	(1.76 - 41.6) 158 ± 133	(0.73 - 41.6) 180 ± 155	(0.73-10.66) $125 \pm 84$	NS; < 0.05, N.S;
	(24 - 380)	(36 - 760)	(31 - 760)	(24 - 760)	(35 - 361)	< 0.05
IgA (I.u./ml)	$206 \pm 92$	$284 \pm 247$	$214 \pm 93$	$254 \pm 233$	$282 \pm 206$	
	(125 - 455)	(112 - 957)	(125 - 554)	(112-1584)	(95 - 957)	NS
IgM (i. U. / ml)	$331 \pm 319$	$160 \pm 80$	$211 \pm 173$	$232 \pm 216$	$130 \pm 47$	< 0.05;NS; <
	(188 - 1148)	(65 - 321)	(88 - 1023)	(65 - 1148)	(69 - 321)	0.05 < 0.05
C3 (% std)	$92 \pm 64$	$110 \pm 59$	119 ±7	$109 \pm 57$	$112 \pm 36$	
	(21 - 290)	(55 - 290)	(70 - 231)	(21 - 290)	(49 - 185)	NS
C4 (% std)	$48 \pm 41$	68 ± 39	53 ± 32	$61 \pm 36$	$43 \pm 25$	P < 0.05; < 0.05
	(11 - 144)	(26 - 144)	(20 - 144)	(11 - 144)	(11 - 98)	NS; < 0.05

or that their activity is blocked by unknown immunosuppressive factors. It is noteworthy that secretory IgA levels in this study were highest in patients with mild to moderate anaemia (with highest eosinophil response and highest worm load), thus indicating that increased levels of secretory IgA are grouped to reduce worm survival and the risk of severe disease.

C4 levels were significantly elevated in hookworm patients with marked anaemia, moderate anaemia and hookworm only (P < 0.05) while C3 levels on the other hand were not significantly different in patients and controls. No significant difference was found in C3 levels between patients and controls in India [3]. The elevated C4 complement levels could be due to a non-specific response to inflammation or stimulation of the classical arm of complement system by formed immune complexes. In patients with inflammatory bowel disease [14], reported rises in C4 levels in the inactive disease have been considered as evidence of reactivation of disease. Hodgson, Potter and Javett [15], also working on the same disease found elevated C4 levels in the active form of the disease. The C4 hyper-complementaemia observed in this study might be due to macrophage induced stimulation and secretion in response to inflammation. Hookworm infection in man has been shown to trigger mast cell degranulation [16] and the interaction of the worms with previously sensitized mast cells bearing IgG causes the release of vasoactive and spasmogenic factors capable of contributing to an increase in local concentration of complement and specific antibody [17]. Also while the increase in C4 levels could reflect a response to an inflammatory state, it could also be a means by which the immune system eliminates or This is suggested by the controls worm antigens. observation that 27 (47%) of the patients in this study who had low egg loads (below 500 eggs per gram of stool) before treatment, had the highest range of complement levels compared to controls (P < 0.05).

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