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Cryoglobulins in *Schistosoma haematobium* infection

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Summary

The sera of school children with *Schistosoma haematobium* infection were tested for the presence of cold-insoluble immune complexes «the cryoglobulins». Two different methods were used: the standard macro-technique and the micro-adaptation technique. On using the standard macro-technique, 40 (32.8%) out of 122 schistosomiasis patients and 6 (7.5%) out of 80 control children were positive for cryoglobulins. Using the micro-adaptation technique, the corresponding numbers were 47 (38.5%) and 8 (10%), respectively. A comprehensive medical examination was carried out before treatment. After treatment with metrifonate (Bilarcil) the quantity of cryoglobulins and the number of children with cryoglobulins were significantly reduced to 12 (9.8%) when using macro-technique, and to 15 (12.3%) with micro-adaptation technique, thus suggesting a possible relationship of cryoglobulinaemia with urinary schistosomiasis. There was a significant difference in the number of sera found positive by the two techniques ($\chi^2 = 0.875$, $P < 0.1$).

Key words: cryoglobulins; *Schistosoma haematobium.*

Introduction

In recent times, evidences are indicating that circulating immune complexes may be involved in the pathogenesis of human schistosomiasis. A form of immune complex glomerulonephritis has been associated with human schistosomiasis and IgG, IgM complexes have been observed in the kidney biopsies of

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these patients (Warren, 1976). Also various immunoglobulins, complement, schistosomal pigments and antigens have been demonstrated by immunofluorescence in the glomerular deposits (Andrade and Susin, 1974; De Brito et al., 1971). Similar findings have been described in mice infected with *S. mansoni* (Natali and Cioli, 1976). These glomerular deposits may cause inflammatory disease condition, and this may account for most cases of human glomerulonephritis.

Cryoglobulins have been observed in sera of patients with glomerulonephritis of the immune complex type (McIntosh et al., 1975), and there are speculations that these cryoglobulins contain circulating immune complexes. The cryoglobulins are abnormal serum proteins or protein complexes which reversibly precipitate at low temperatures. They are formed as a result of immune responses to various antigens.

There are three types of cryoglobulins (Stite, 1980) which are:
- Type I: the monoclonal cryoglobulin,
- Type II: the mixed cryoglobulin, and
- Type III: the mixed polyclonal cryoglobulin.

Types II and III cryoglobulins have been indicated to be examples of soluble immune complexes (Brouet et al., 1974) and the hypothesis that they contain immune complexes, is supported by observations describing the presence of antigens and specific antibody in cryoglobulins (Griswold and Brady, 1978). Also, patients with systemic lupus erythematosus have cryoglobulins containing DNA antigen and anti-polynucleotide antibody (Winfield et al., 1975). Strife et al. (1976) reported a finding of bacterial antigen and antibody in a cryoglobulin isolated from a patient with shunt nephritis.

The present study is dealing with cryoglobulin in its formation in *Schistosoma haematobium* infection, and its relationship with morbidity. Also, a trial of micro-adaptation technique (Adeiga and Ade-Serrano, in press) in the determination of cryoglobulins is made. This technique is adopted along with the standard macro-technique, so as to enable us to know which method will be more acceptable for field use. Finally, the effect of treatment with metrifonate on the formation and/or quantity of cryoglobulins such as Penicillamine (Deutsch and Morton, 1957) has been studied.

Patients, Materials and Methods

Patients

122 school children from a village in Lagos State, Nigeria with *Schistosoma haematobium* infection were the study group; details of which were reported elsewhere (Ejezie and Ade-Serrano, 1981). The intensity of infection determined by urine egg counts, using the filtration technique varied from light to heavy infection (Ejezie and Ade-Serrano, 1981). Their ages ranged from 6 to 14 years. 80 school children from the same school and living in the same village who had no *Schistosoma* infection, served as controls.

Table 1. Cryoglobulinaemia in *Schistosoma* patients and in controls, using the standard macro-method and micro-adaptation method

	Children with schistosomiasis		Controls
	Before treatment	After treatment	
Number of subjects	122	122	80
Micro method: number of positive sera	47 ± 5.23* (38.5)**	15 ± 2.6 (12.3)	8 ± 1.2 (10)
Standard method: number of positive sera	40 ± 5.23 (32.8)	12.2.1 (9.8)	6 ± 1.04 (7.5)
Cryocrit values (mean)	6.8 mm	3.9 mm	4.1 mm

* Mean values ± standard deviation.
** Figures in brackets are percentages.

Serum samples

Blood was collected in a warm syringe from the patients and the controls, and was kept at 37° C until it clotted. Sera were separated from the blood samples by centrifugation at 1500 × *g* for 15 min at 37° C and they were tested within 48 h to prevent cholesterol crystals from precipitation in hyperlipaemic sera.

Detection of cryoglobulins

Two methods were used to detect the cryoglobulins in the serum samples: the standard macro-test (François et al., 1978), and the micro-capillary adaptation method (Adeiga and Ade-Serrano, in press).

Standard macro-test. 1 ml of each serum sample was measured into sterile test tubes and incubated at 37° C for 2 h in a thermostatically controlled water bath. This was because of the room temperature (22° C–24° C) wich the samples were formerly subjected to. Immediately after removal from warm water bath, they were incubated again at 4° C for 24 to 72 h. The sera were examined by indirect light for the presence of precipitate or gel which denoted the presence of cryoglobulins. Absence of precipitate was taken as negative. When precipitation did occur, this was washed with 1.5 ml of buffered saline (PBS), pH 7.2, by agitation and centrifugation at 1500 × *g* for 15 min. The precipitate was then resuspended in 0.1 ml of PBS and incubated at 37° C for 30 min. Redissolution of the precipitate confirmed cryo-precipitation. Sera with insoluble precipitates were discarded.

Micro-capillary adaptation method. Sera were filled into two 50 µl capillary tubes (non-heparinised). Both ends were sealed with sterile non-toxic plasticine. The capillary tubes were fixed on glass slides with thin stripes of adhesive and placed in a water bath at 37° C for 2 h and later at 4° C for 24 to 72 h. Each capillary tube was scanned under light microscopy × 4 objective and × 10 eye piece to examine for cryoprecipitates. The precipitates were centrifuged at 1500 × *g* for 15 min and cryocrit was determined by linear microscopic measurements. Reincubation of the capillary tubes at 37° C was done and redissolution of precipitate confirmed crypoprecipitation.

The two test procedures, the macro- and micro-methods were again repeated two weeks after treatment of the *Schistosoma* positive patients with metrifonate (10 mg/kg) in a single dose.

Clinical examination

On each child, physical examination was carried out. This included the palpation of the liver and spleen and anthropometric measurements (head and mid-arm circumferences, height and

weight). Urine analysis was carried out on the whole sample immediately after urine was voided by means of Bili-Labstix reagent strips, and the amount of protein and blood in the urine was recorded. Laboratory tests such as haematocrit, malaria parasitaemia (microscopic counts) AB0 blood grouping and Rh factors were also carried out (Ejezie and Ade-Serrano, 1981).

Results

Using the standard macro-test before treatment, cryo-precipitate was detectable in 40 (32.8%) out of 122 sera of school children with *Schistosoma haematobium* infection. Also 6 (7.5%) of the 80 serum samples from the control group were positive with the standard macro-test. The results of the micro-adaptation test are shown in Table 1. The difference in the number of positives between the two tests was found to be significant ($\chi^2 = 0.875$, $P < 0.1$).

After treatment with metrifonate, the number of positives and the quantity of cryoprecipitates formed were reduced (Table 1). The reduction was found to be significant both with the macro-technique ($\chi^2 = 0.67$, $P < 0.1$) and the micro-adaptation technique ($\chi^2 = 0.638$, $P < 0.1$). Tests with three sera found negative by the macro-test, but positive by micro-capillary method were repeated and the same results were obtained. The results of the clinical aid laboratory investigations are shown in Table 2.

Discussion

The formation of soluble antigen from worms and eggs and the detection of corresponding antibodies in subjects with schistosomiasis, may lead to the formation of immune complexes (WHO, 1974). Soluble immune complexes may be formed from these antigen antibody reactions, which may be capable of giving rise to a serum-sickness like picture (Gold et al., 1969; Bawden and Weller, 1974) wich cryoglobulins cause.

In this study, the formation of cold precipitable immune complexes, the «cryoglobulins», which are found in immune complex diseases in man (Brouet et al., 1974; Winfield et al., 1975) was investigated in *Schistosoma haematobium* infection. The possibility of their formation was suspected in view of possible soluble antigen-antibody reaction that could be formed in *Schistosoma* infection (WHO 1974), and in view of the clinical picture manifested in the course of the disease.

The findings of proteinuria and haematuria observed in these school children with schistosomiasis (Table 2) (Ejezie and Ade-Serrano, 1981) may indicate renal dysfunction with glomerular involvement. They are known to be associated with heavy *S. haematobium* infection (Forsyth and Bradley, 1966; Lehman et al., 1975). Symptoms like splenomegaly, hepatomegaly, skin rash, urticaria, and fever (Table 2) are found in primary schistosomiasis, shortly after the egg-laying by the worms (Lawley et al., 1979). Warren (1976) has suggested

Table 2. Clinicopathological findings of school children with schistosomiasis and controls before and after treatment with metrifonate

Clinico-pathological findings	Before treatment		After treatment	
	Schistosomiasis positive (n = 122)	Controls (n = 80)	Schistosomiasis positive p(n = 122)	Controls (n = 80)
Fever*	70 (57.4)**	25 (31.2)	29 (23.8)	25 (31.3)
Hepatomegaly	20 (16.4)	2 (2.5)	17 (13.9)	2 (2.5)
Splenomegaly	16 (13.1)	1 (1.2)	11 (9.0)	1 (1.3)
Skin rash	45 (36.9)	0 (–)	43 (35.2)	0 (–)
Urticaria	7 (5.7)	2 (2.5)	9 (7.4)	2 (2.5)
Haematocrit (mean ± SD)	33 ± 2.7	34.1 ± 1.9	35.2 ± 1.8	33.9 ± 2.1
Proteinuria	49 (40.2)	0 (–)	2 (1.6)	0 (–)
Haematuria	44 (36)	1 (1.3)	3 (2.5)	1 (1.3)
Malaria parasite	49 (40.2)	23 (28.8)	40 (32.8)	21 (26.2)
Mean *S. haematobium* egg count	459.7	Nil	8.1	Nil

* Most children with fever had malaria parasite in the thick blood films.
** All figures in brackets are percentages.
n = number of subjects.

that the pathogenesis of these symptoms may be associated with the formation of circulating immune complexes.

Circulating immune complexes have been demonstrated in patients and animals with schistosomiasis (Berggren and Weller, 1967; Smith et al., 1975; Voller et al., 1976). This has led to the hypothesis of an immune complex nephritis to occur in schistosomiasis patients.

One mechanism of glomerular disease in infection is the production of antibodies, which are capable of reacting with soluble antigens in the circulation to form soluble circulating antigen-antibody complexes which are subsequently trapped in the glomerular capillary wall (Hoshino-Shimizu et al., 1975). This appears to be the mechanism of renal injury in chronic infectious diseases like schistosomiasis, malaria and Kala-azar (Kibukamusoke, 1973; De Brito et al., 1975) where the circulating immune complexes are presented over a long period to the kidney, eventually producing glomerular disease characterised by various clinical and laboratory features. These range from slight proteinuria, with preservation of renal function (Da Silva et al., 1970), to advanced clinical disease (De Brito et al., 1970; Lehman et al., 1975).

McIntosh et al. (1975) indicated that cryoglobulins do contain immune complexes which may be of immunopathologic significance. Their observation of cryoglobulins in sera of patients with glomerulonephritis of the immune complex type, suggest the involvement of cryoglobulins in the pathogenesis of renal disease. Thus the cryoglobulins detected in the sera of children in our study might contribute to the pathogenesis of the infection through the immune

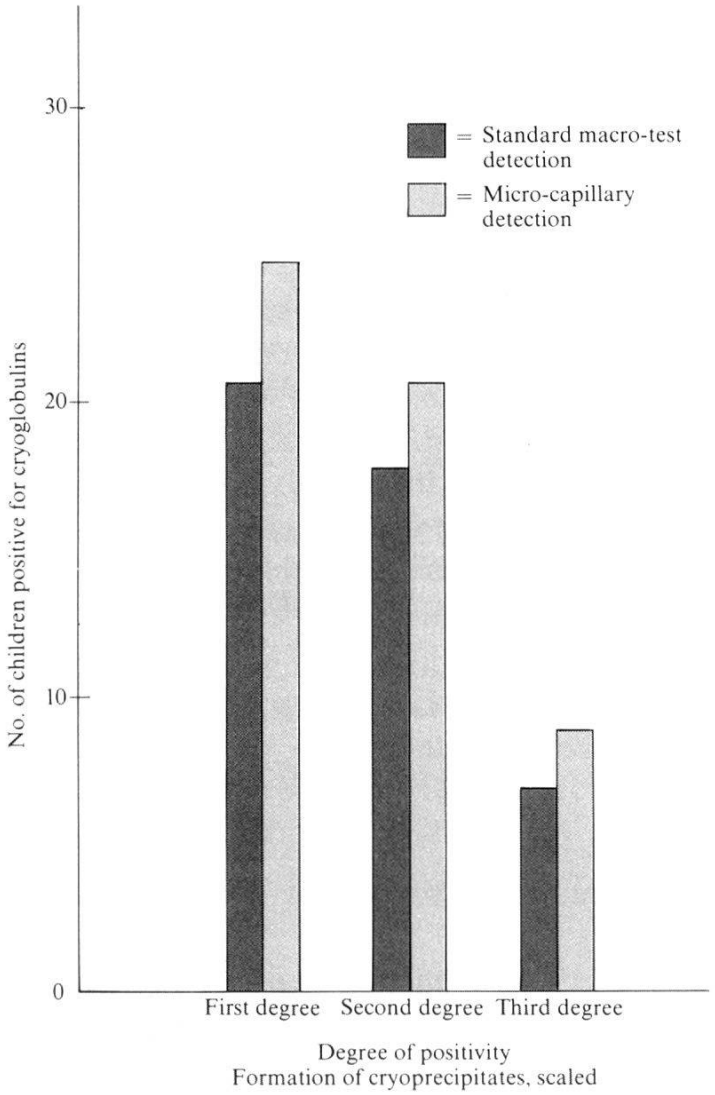

Fig. 1. Relationship between standard macro-method and micro-capillary method.

complexes they might contain. This was in view of the high level of proteinuria (Ejezie and Ade-Serrano, 1981) which is strongly suggesting glomerular damage. Other possible pathogenic roles of cryoglobulins are complement activation (Muller et al., 1976), platelet aggregation (Cortellaro et al., 1975), cytotoxic activity (Winfield et al., 1975), or saturation of the reticulo-endothelial system. When the protective capacity of the spleen, liver and mesangial cells which normally remove large protein complexes from circulation is overwhelmed, deposition of these complexes may occur in the glomerular capillaries (Griswold and Brady, 1978) and cause glomerular damage.

Some of the symptoms found in children with *S. haematobium* infection were observed in some of the control children too. This might be attributable to malaria which is endemic in the area, and may induce cryoglobulin production. This is in view of circulating antigen-antibody complexes that have been demonstrated in sera of malaria patients (Smith et al., 1972).

In the two methods adopted for the study, the micro-adaptation method

could be more acceptable to the children than the standard macro-test, since the former can be done with small amount of sera obtained by finger-prick. It is also easier to determine the cryocrit with micro-adaptation technique.

The difference observed in the two methods might be attributable to difference in cohesive forces in the immune complex formation.

The detection of cryoglobulin in both the children with schistosomiasis and the control group indicates that cryoglobulins can occur in most infectious diseases.

The complication of the disease by cryoglobulins through immune complex formation, especially when the kidney is involved should be viewed critically.

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