

THE CIRCUMOVAL PRECIPITIN TEST AS A CONTROL OF CURE IN CHILDREN WITH CHRONIC SCHISTOSOMIASIS MANSONI (1)

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S U M M A R Y

Seventy nine children in the 6-14 age group, with chronic schistosomiasis mansoni were selected for this study. Sixty seven (85%) with positive circumoval precipitin test (COPT) were divided in two groups: 36 children (**Group 1**) were treated with **oxamniquine** (20 mg/kg in a single oral dose) and 31 children (**Group 2**) were given placebo under the same conditions. Follow-up was maintained for 10 months with monthly quantitative stool examinations and the absence of fecal eggs from the first month onwards was considered as a successful treatment. COPT was repeated on the tenth month. In **Group 1**, 69% were cured. **Group 2** showed no cures. In the 23 **Group 1** patients considered cured, COPT was negative in 20 (87%). Negative reactions were also found in 32% of the **Group 2** children, after 10 months. These false negative reactions occurred in patients with a smaller stool egg count. The Authors conclude that COPT shows a high positivity ratio in the diagnosis of chronic schistosomiasis mansoni, with negative reactions post cure in the majority of cases. However, a significant number of false negative results does not allow the isolated use of COPT in the control of cure of schistosomiasis mansoni.

I N T R O D U C T I O N

OLIVER-GONZALEZ¹⁰, observed that the incubation at 37°C of a mixture of *S. mansoni* eggs with serum from patients with schistosomiasis, led to the formation of globule or chain like precipitates similar to *Taenia* segments around the eggs. These precipitates were shown to be antigen-antibody complexes⁴, involving gGI, IgM and IgA³.

The value of COPT in the diagnosis of schistosomiasis has been investigated by many Authors^{2,8,18}. Its sensitivity is around 90% and can be increased by immunofluorescence techniques¹².

The possibility of using COPT as a control of cure in schistosomiasis has been suggested^{9, 11,14,15}. Serum reactions after specific treatment shows a lowering of titres after 60 to 120 days, becoming negative in most patients between 6 to 10 months after cure. The value of COPT in the control of cure however, has not been adequately established. Some Authors^{16,17}, have observed that positive reactions remain unchanged for more than 10 months after successful treatment.

The present work evaluates the use of COPT as a control of cure in children with chronic schistosomiasis mansoni, treated with **oxamniquine** 20 mg/kg in a single oral dose.

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MATERIAL AND METHODS

Studied population — The children were selected for this study through a coprological survey in a grammar-school located in the periphery of the metropolitan area of Belo Horizonte (Brazil). Quantitative stool examinations⁵ were performed in 1500 children within the age range of 6 to 14 years. *S. mansoni* eggs were found in the feces of 120 children (8%). Following initial selection, parents were informed of the aims of this study and permission was obtained for the treatment of 79 children. They were followed up post-treatment at the out-patient clinic or at home. Stool samples were collected with the help of the school teachers and/or the health assistant⁷.

Seventy nine children underwent COPT prior to treatment and only the positive cases were followed up in the study. Thus, 67 children with chronic schistosomiasis mansoni were divided in 2 groups:

Group 1 — 36 children were given **oxamniquine**, 20 mg/kg in a single evening oral dose;

Group 2 — 31 children received the placebo under similar conditions as **Group 1**.

At the end of the study, **Group 2** children were given **oxamniquine** as **Group 1** children. Table I shows the general characteristics of the studied population.

T A B L E I

Distribution of patients in Groups 1 and 2, according to number, age, sex, clinical form of schistosomiasis and oral dose of oxamniquine

Groups	Number of patients	Sex		Age (years)	Clinical form (*)		Oral dose of oxamniquine (mg/kg)
		M	F		HI	HS	
1 (oxamniquine)	36	25	11	6-14 (mean: 10.9)	32	4	20 (17.5-24.5)
2 (placebo)	31	16	15	6-14 (mean: 10.7)	30	1	—

(*) HI: Hepatointestinal form

HS: Hepatosplenic form

Another group (**Group 3**) comprising 45 children in the 8-14 age group, without schistosomiasis mansoni, and living in the same region were used as control of the disease transmission in the studied area. All of them had two negative stool examinations and two negative immunological tests (complement fixation reaction and immediate skin test with adult schistosome antigen). The serum of these children were also tested with COPT.

Cure control — The children have been followed up for 10 months with monthly quantitative stool examinations (KATO'S method modified by KATZ et al.⁵). Two consecutive exams were performed each month, with 2 slides for each stool sample. The average egg count of the 4 slides was considered each month. The absence of fecal eggs from the first month onwards was considered as a successful treatment. **Group 3** children undertook two stool examinations on the 3rd, 5th, 9th and 12th months. On the 12th month, 3 immunological

tests were performed (complement fixation reaction, skin test and circumoval precipitin test).

COPT, Complement fixation reaction (CFR) and Skin test (ST) — The circumoval precipitin test according to the OLIVER-GONZALEZ¹⁰ technique modified by MELLO et al.⁹, using fresh *S. mansoni* eggs obtained from the liver of infected hamsters, was performed in **Groups 1, 2** and **3** children in the beginning of the study, and repeated on month 10 post-treatment in children of **Groups 1** and **2**. Blood samples were promptly transferred to a centrifuge tube, frozen at -20°C and tests were performed within 1 to 3 months after serum sample collection. Results were considered as positive or negative and were interpreted by one of the Authors (RTM) in a single blind manner. The skin test and complement fixation reaction followed the technique described by PELLEGRINO¹².

Statistical analysis — For the nominal variables, chi-square (X)² test was used. For the

intervalar variables the student 't' test for non correlated samples was employed. It was accepted, for all tests, the significance level of 5%.

RESULTS

Sixty-seven out of 79 (85%) children with chronic schistosomiasis mansoni, presented positive COPT at the beginning of the study. Twenty-five out of 36 (69%) **Group 1** children treated with **oxamniquine** were cured according to our parameters. No **Group 2** children were cured. Of the 25 **Group 1** cured patients, 2 did not undertake the COPT on the tenth month of follow-up, and therefore were not considered in the results. Thus, from 23 cured patients, only 3 did not have negative tests post-treatment, i.e., 87% of the patients presented a change from positive to negative tests after cure. Ten out of 31 (32%) **Group 2** children also presented negative COPT on the tenth month of follow-up. In 21 **Group 2** patients with positive COPT on the tenth month of follow-up, the average egg count per gram of feces was 594, compared to an average of 66 eggs/gram of feces in 10 children of the same group with negative COPT ($p < 0.05$).

On the remaining 11 **Group 1** patients not cured with **oxamniquine** a 70% reduction in the number of fecal eggs as compared to pre-treatment counts was observed. COPT was negative in 5 (45%) of these patients. This negative percentage in **Group 1** compared to **Group 2** on the tenth month was not statistically significant at the 5% level.

COPT was negative in all the 45 **Group 3** children without schistosomiasis mansoni at the beginning of the study. On the 12th month of follow-up *S. mansoni* eggs were found in the feces of 2 children (4.4%). COPT and CFR became positive at this time.

DISCUSSION

In schistosomiasis mansoni, when the patient carries a small number of worms, with a resulting low stool egg count, parasitological diagnosis becomes difficult. Since 1954, various studies have shown that the circumoval precipitin test provides good results in the diagnosis of this disease^{1,13,16}. Our cases showed a posi-

ve reaction in 85% of 79 patients with *S. mansoni* eggs in the stool.

Among other factors which could be considered as responsible for false negative COPT is the hemolysis of red blood cells and the resulting presence of haemoglobin in the serum⁸. Storage time prior to reading is also significant, since antiserum gradually loses its titre even at low temperatures. WOODHEAD¹⁹ showed a reduction of titre in the complement fixation reaction directly proportional to storage time and temperature. This is also noticed with COPT antibodies, with a reduction in the reaction intensity for each month of storage at -20°C⁸. KLOETZEL⁶ observed a direct relationship between reaction intensity and the number of infecting parasites. In our qualitative readings it has not been possible to confirm this information. However, it is reasonable to think that patients with low stool egg counts would present a higher number of false negative reactions. Analysis of our cases shows that in patients with negative COPT, the average egg count per gram of feces is significantly lower than those with positive COPT ($p < 0.05$).

OLIVER-GONZALEZ et al.¹¹ introduced COPT as a cure control in schistosomiasis mansoni. Fifteen patients with positive reactions were given an antimonial drug (Fuadin[®]). Thirteen (87%) of them had negative COPT between 120 and 183 days post-treatment. Our results agree partially with those described by the quoted Author. From 23 cured patients, 20 (87%) presented negative reactions on the tenth month post-treatment. On the other hand, 10 out of 31 **Group 2** children (32%) also presented negative reactions on the 10th month of follow-up. This number of false negative reactions in the placebo group questions the reproducibility of the reaction and its validity in the evaluation of cure of schistosomiasis mansoni. Considering that the reaction tends to negativity in cases with low stool egg counts, as suggested in this study, and also that storage time may reduce its intensity, these facts may partially explain our negative results in the placebo group and in the **Group 1** not cured cases.

The Authors conclude that the circumoval precipitin test shows high positivity ratio in the diagnosis of chronic schistosomiasis mansoni, and that the reaction becomes negative in most treated and cured cases. The large num-

ber of false negative reactions in this study does not allow the isolated use of COPT in the control of cure of this disease.

RESUMO

A reação periovular como controle de cura em crianças com esquistossomose mansoni crônica

Setenta e nove crianças com esquistossomose mansoni crônica, na faixa etária de 6-14 anos, foram selecionadas para o estudo. Sessenta e sete (85%), com reação periovular positiva, foram divididas em dois grupos: 36 crianças (**Grupo 1**) foram tratadas com a oxamniquine oral (20 mg/kg de peso, dose única) e 31 (**Grupo 2**) receberam o placebo nas mesmas condições do **Grupo 1**. Após o tratamento, as crianças dos dois grupos foram acompanhadas durante 10 meses com exames de fezes quantitativos mensais e no 10.º mês repetiu-se a reação periovular. O índice de cura foi de 69% no **Grupo 1**. Nenhuma criança se curou no **Grupo 2**. Em 23 pacientes considerados curados no **Grupo 1**, a reação periovular foi negativa em 20 (87%). Houve também negatificação da reação periovular, no 10.º mês, em 32% das crianças que receberam o placebo. As reações falso-negativas no **Grupo 2**, ocorreram nos pacientes que eliminavam menor número de ovos nas fezes.

A reação periovular mostra alto índice de positividade no diagnóstico da esquistossomose mansoni crônica em crianças e ocorre negatificação da reação na maioria dos pacientes tratados e curados. O grande número de reações falso-negativas, encontradas no presente estudo, entretanto, desaconselha o uso isolado da reação periovular no controle de cura da esquistossomose mansoni.

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