

## A PERORAL BIOPSY STUDY OF THE JEJUNUM IN HUMAN SCHISTOSOMIASIS MANSONI

Luiz de Paula CASTRO, Renato DANI, Roberto Junqueira ALVARENGA, Dalton de Alencar Fischer CHAMONE and Celso Affonso de OLIVEIRA

### SUMMARY

The involvement of the small intestine in human schistosomiasis mansoni was studied in 100 patients by peroral jejunal biopsy. Thirteen were in the initial period of the infection, 43 had the intestinal form and 44 the hepatosplenic. The compression preparation technique were used in all cases, for the searching and counting of the schistosomotic elements. In 52 cases, a fragment of jejunal mucosa was sent for histological examination, of which only 35 were considered adequate for histological analysis and classification. The dissecting microscope examination was done in 74 cases for analysis and classification of the intestinal villi, and rectal biopsies were carried out in 92 patients for comparative study.

The jejunal peroral biopsy showed schistosomotic elements in 43 out of the 100 patients, having been positive in 69.2% of the cases in acute phase, 25.6% in those with the intestinal form and in 52.3% of those with hepatosplenic form.

The rectal biopsy, was positive in 80.4% of which 90.9% were in the acute phase, 95.0% with intestinal form and 63.4% with hepatosplenic form.

The histological examination showed mild and nonspecific alterations in most of the cases, being negative for schistosomotic elements.

The intestinal villi did not differ significantly from the pattern we have seen in patients without schistosomiasis.

The data obtained show that the upper jejunal mucosa is a frequent site for oviposition in the acute as well as in the hepatosplenic form of schistosomotic infection, more than the histological examinations alone reveal.

The peroral biopsy, with direct microscopy of compression preparation of the fragment proved to be a valuable method for study of the involvement of the small intestine in the human mansoni schistosomiasis.

### INTRODUCTION

The involvement of the small intestine in human schistosomiasis has been the subject of very few studies. The data obtained up to now can be summarized, practically, in those found in autopsies<sup>2, 11, 13, 14, 22</sup>. The light infections and the lack of specific ab-

normalities found by histological examinations have contributed, to a certain extent, to the paucity of information about the involvement of the small intestine in the infection caused by the *Schistosoma mansoni* in man. CHEEVER<sup>6</sup>, based on data from autopsies and di-

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Departments of Medicine and Pathology, University of Minas Gerais School of Medicine, Belo Horizonte, Minas Gerais, Brazil

Address request for reprints to: Luiz de Paula Castro. Departamento de Clínica Médica, Faculdade de Medicina da UFMG. Hospital das Clínicas, Belo Horizonte, Minas Gerais, Brasil

rect microscopy examination showed that this segment of the digestive tract can be greatly affected, depending on the degree of parasitism and on the clinical form of the disease.

The peroral jejunal biopsy has made it possible to study, histologically and morphologically, the intestinal mucosa in different diseases of the small intestine. This procedure has been used, also, to demonstrate the tissue alterations caused by the larvae and eggs of various helminths, in patients with intestinal parasitosis. However, the finding of schistosomotic elements (viable and dead eggs, calcified eggs, shells or granulomas) in fragments of jejunal mucosa, has been relatively rare<sup>10</sup>.

This paper, an amplification of the studies initiated by CASTRO & DANI<sup>5</sup> shows the results obtained by jejunal peroral biopsy, with direct microscopy (compression preparations) and histological examination of fragments, taken from patients with the various clinical forms of schistosomiasis.

#### MATERIALS AND METHODS

One hundred patients with active schistosomiasis mansoni were examined. Thirteen were in the initial periods of the infection, classified under the acute form<sup>9, 18, 43</sup> had the intestinal form, also known as hepatointestinal, and 42 had the hepatosplenic form, according to the criteria suggested by DIAS<sup>8</sup> and MEIRA<sup>15</sup>. Seventy three cases were males, and the others 27 females. There were 16 children and 84 patients older than 14 years of age.

The jejunal peroral biopsy was done with the ROY-CHOUDHURY et al. capsule<sup>20</sup>, slightly modified by CASTRO<sup>4</sup>. The fragment of jejunal mucosa were nearly always taken at the Treitz angle, in the upper portion of the jejunum. These fragments, whenever possible, were divided into two parts by a thin, cutting blade, immediately after the entire fragment had been studied by a dissecting microscope, in order to classify the intestinal villi. A part of this fragment was sent for histologic examination; the other one was crushed between glass slides and examined microscopically for searching and counting of schistosomotic elements. The ROY-CHOUDHU-

RY et al. classification<sup>21</sup> was adopted for the histological analysis. These Authors classify the jejunal mucosa into normal, Grade I, Grade II and Grade III abnormalities, according epithelium, the intensity of the inflammatory infiltration and the thickness of the glandular mucosa.

Only 52 out of 100 jejunal biopsies were histologically examined. The remaining<sup>48</sup>, because the fragments were quite small, were only for a parasitological analysis using the compression preparation, in which the fragments were crushed between glass slides and examined. In 42 patients, jejunal fluid was obtained during the suction done with the siringe, for collecting the jejunal mucosa. This fluid was centrifuged and examined in the microscope, in searching for *S. mansoni* eggs.

The rectal biopsy was done immediately before or after the jejunal biopsy in 92 patients there being 11 with the acute, 40 with the intestinal and 41 with the hepatosplenic form. The oogram was done according to Prata's criteria<sup>19</sup>. For the statistical analysis, STUDENT'S "t" test was used.

#### RESULTS

##### a) *Parasitological analysis of fragments of jejunal and rectal mucosa examined by the compression preparation*

The jejunal peroral biopsy was positive for schistosomotic elements, in 43 patients, that is, in 43% of the cases, 9 (69.2%) with the acute, 11 (25.6%) with the intestinal and 23 (52.3%) with the hepatosplenic form (Table I). The rectal biopsy, done in 92 patients, was positive for schistosomotic elements in 74 (80.4%), being positive in 90.9% of those with the acute, 95.0% with the intestinal and in 63.4% with the hepatosplenic form (Table I).

On the other hand, viable eggs found in the positive jejunal biopsies in 88.9% with the acute form, 18.2% with the intestinal and 52.2% with the hepatosplenic form. In the rectal biopsies viable eggs were found in 90.0% with the acute form, 97.4% with the intestinal and 46.2% with the hepatosplenic form (Table II).

TABLE I

Percentage of positivity of jejunal and rectal biopsies from 100 cases of schistosomiasis mansoni

Clinical form of schistosomiasis	Jejunal biopsies %	Rectal biopsies %
Acute	69.2	90.9
Intestinal	25.6	95.0
Hepatosplenic	52.3	63.4
Total	43.0	80.4

The statistical analysis showed a significant difference between the incidence of schistosomotic elements, in the jejunal biopsies of the patients with the hepatosplenic form (52.3%) when compared with those with the intestinal form (25.6%) ( $t > 2$ ,  $\alpha = 5\%$ ).

The differences found between the acute and the hepatosplenic form and between the acute and the intestinal form were not statistically significant.

The suction made with the siringe during the biopsy procedure, brought up some jejunal fluid, collected inside the siringe, in 42 patients, the volumes ranging from 3.5 to 8.5ml. The direct microscopic examination of this material after centrifugation, showed *S. mansoni* eggs in 16 (38.1%), although schistosomotic elements in the jejunal biopsy fragments were found in 23 (54.8%). Nevertheless there were four cases with eggs in jejunal fluid without the corresponding schistosomotic elements in the fragments of jejunal mucosa (Table III). Two of the four cases had the intestinal form and others two the hepatosplenic. These four cases were not considered for the global analysis of this paper, prevailing only the findings in jejunal mucosa.

b) *Morphological and histological analysis of fragments of the jejunal mucosa*

Only 35 of the 52 jejunal biopsy fragments examined histologically were considered adequate for histological analysis and classifications. Seven biopsies were considered as fitting the normal pattern; 23 classified as Grade I abnormalities and 5 as Grade II. None

TABLE II

Incidence of schistosomotic elements in jejunal and rectal biopsies from patients with acute, intestinal and hepatosplenic forms of schistosomiasis mansoni

Schistosomotic elements	Acute		Intestinal		Hepatosplenic	
	Jejunal biopsy %	Rectal biopsy %	Jejunal biopsy %	Rectal biopsy %	Jejunal biopsy %	Rectal biopsy %
Viable eggs	33.3	10.0	0.0	2.7	17.4	7.7
Dead eggs and/or granulomas and/or shells	11.1	10.0	81.8	2.7	47.8	53.8
Viable and dead eggs and/or granulomas and/or shells	55.6	80.0	18.2	94.6	34.8	38.5
Total	100.0	100.0	100.0	100.0	100.0	100.0

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TABLE III

Incidence of schistosomic elements in the jejunal fluid and jejunal biopsy in 42 patients

Clinical form	Positivity of jejunal biopsy and intestinal fluid				Total
	Biopsy (+) Int. fluid (+)	Biopsy (+) Int. fluid (+)	Biopsy (+) Int. fluid (+)	Biopsy (+) Int. fluid (+)	
Acute	4	4	—	2	10
Intestinal	—	3	2	6	11
Hepatosplenic	8	4	2	7	21
Total	12	11	4	15	42

of the biopsies were classified as Grade III abnormalities (Table IV).

In none of these 52 fragments, examined histologically, were any schistosomic elements found.

The dissecting microscope examination was done in 74 cases. The great majority show finger and leaf-shaped villi and, in a small number, ridges and partially convoluted ones. No totally convoluted villi or flattened mucosa were observed.

#### DISCUSSION

The analysis of the incidence of schistosomic elements in the jejunal biopsies (43.0%) compared with the incidence in rectal biopsies (80.4%) is consistent with the view that, for the practical standpoint, that is, for the diagnosis of individual cases and control of cure of the mansoni schistosomiasis, the rectal biopsy continues to be the preferable procedure, especially in the demonstration of viable eggs (Table I and II).

TABLE IV

Results of histological reports of jejunal biopsies according to the classification of Roy-Choudhury et al., done on 35 patients with schistosomiasis mansoni

Histological classification	Clinical form of schistosomiasis			
	Acute	Intestinal	Hepatosplenic	Total
Normal	—	5	2	7
Grade I	5	10	8	23
Grade II	—	1	4	5
Grade III	—	—	—	—
Total	5	16	14	35

The high incidence of schistosomotic elements (69.2%) in the acute form of mansoni schistosomiasis, with high prevalence of viable eggs (88.9%) justifies the assumption that the eggs, in the acute form of the disease, are laid with great frequency and continuously in the submucosa of the small intestine. The oviposition during the acute phase of the mansoni schistosomiasis seems, therefore, to be done in other layers of the small intestine and not only in the subserous as had already been reported<sup>1, 3, 7, 9, 16, 18</sup>.

In those patients with the intestinal form, without hepato and splenomegaly, with no signs of portal hypertension and hepatic fibrosis, the jejunal biopsy was positive for schistosomotic elements in 25.6% with only 18.2% of viable eggs, contrasting clearly with the 95.0% of positivity of the rectal biopsy in the same group, with 97.4% of viable eggs. These findings are in close agreement with those found by other Authors,<sup>1, 13, 17, 19, 22</sup> showing that the oviposition in the intestinal form of schistosomiasis is especially in the distal portions of the gut<sup>15</sup>.

In those jejunal biopsies made on patients with the hepatosplenic form there was an incidence of 52.3% of schistosomotic elements with 52.2% viable eggs. In the same group, the incidence of schistosomotic elements in the rectal biopsies was of 63.4% which only 46.2% of viable eggs. These findings shows that in patients with the hepatosplenic form of the disease the eggs are laid mostly on the upper portions of the gut in contrast with those with the intestinal form, where they are laid mostly on the distal segments. There is, therefore, evidence to justify the assumption that the oviposition in the jejunum is more frequent in those patients with hepatic fibrosis than in those without. Our data seems to confirm the autopsies findings of CHEEVER<sup>6</sup>, when it was shown that in Symmers fibrosis the oviposition is heavier in the transverse colon and in the jejunum than in other segments of the gut.

The data here obtained, together with those found in the literature, allow us to argue some theoretical and practical aspects of the pathogenesis of schistosomiasis mansoni in man. Since schistosomiasis is a parasitosis essentially of the portal vein system, it must

be admitted that the organs that belong to it must be the site for the oviposition of the *S. mansoni* females. Therefore, one expects to find schistosomotic elements in the wall of the esophagus, stomach, small and large intestine, liver, pancreas, spleen and the biliary tract<sup>11, 14, 22</sup>. Corroborating this statment is the fact that in two autopsied cases of hepatosplenic schistosomiasis mansoni, seen by one of us, the search for schistosomotic elements in the digestive tract, by the compression preparation technique, shown that *S. mansoni* eggs were laid on the mucosa layers of terminal esophagus, stomach and of the entire small and large intestines (unpublished data). What is unusual, however, is to find schistosomotic elements outside the portal vein system, which should only happen when there is some anatomical abnormalities, as occur in cases of deep functioning colateral circulation, due to portal hypertension.

A correlation among the number of eggs laid in the small intestine, the intensity of the parasitism and the degree of portal hypertension has been postulated by some Authors. KOPPISCH<sup>13</sup> using autopsy material, was able to show the small intestine were less involved in mild (95%) than in heavy infection by the *S. mansoni*. VALLADARES<sup>22</sup> reported the involvement of the small intestine in 25 out of 45 post-mortem examinations from schistosomotic patients, correlating the involvement of the intestine with the degree of the infection. CHEEVER<sup>6</sup> found on the other hand a higher incidence of *S. mansoni* eggs in the small intestine when compared with the rectosigmoid in cases with portal hypertension, the opposite occurring in those cases without portal hypertension. We obtained similar results, suggesting that there actually is a greater oviposition in the small intestine of patients with hepatic schistosomotic fibrosis. This situation also occurs in cases of hepatic cirrhosis, without Symmers' fibrosis, and with very scarce parasitism. The local venous shunts between the inferior cava and the portal system (rectal varices due to hypertension of the superior haemorrhoidal vein, tributary of the inferior mesenteric and in communication with the inferior and media haemorrhoidal and afluent of the system of the inferior vena cava) seems to

be the common causes of these phenomena which occurs in two otherwise different liver diseases.

Schistosomotic elements were not identified in the 52 jejunal mucosa fragments that were histologically examined, though *S. mansoni* eggs were found through direct microscopy in most of them. This finding shows that the parasitism which has been found in various parts of the human body must be a great deal more intense than the solely histological studies demonstrate. Even though the data already published, originating from autopsies, are exclusively based upon histological examinations and tissue digestion by KOH<sup>2</sup> the absence of schistosomotic elements by these methods does not exclude the parasitism by the *S. mansoni*. The systematized study, using the compression preparation on fragments crushed between glass slides, a part of being a simple method, will bring a valuable contribution to the more detailed knowledge of the extension and intensity of the parasitism in the schistosomiasis mansoni infection in man.

The histological alterations found in the fragments of jejunal mucosa were of mild intensity in great majority of the cases, and totally nonspecific. Since most of the patients also suffered from other parasitoses and/or malnutrition, it is impossible to attribute the alterations found to the schistosomotic infection alone. HALSTED et al.<sup>12</sup>, having studied the jejunal biopsies of 10 schistosomotic patients, reported results quite similar to those mentioned here.

The compression preparations of intestinal mucosa permit the study of the parasitism of the small intestine by *S. mansoni*. It became evident that the proximal jejunum is more frequently infected in the patients with the hepatosplenic form than in those with the intestinal form. The data taken from literature suggest that the more intense parasitism and the portal hypertension are the prominent determining factors. The involvement of the small intestine, in the early stages of the schistosomotic infection is found with great frequency, not only in the intestinal mucosa as well as in the subserous. However, more clinical and parasitological data are necessary for its better understanding. The histological analysis of the jejunal mu-

cosa did not bring any contribution to the diagnosis of the parasitosis, when compared with the data obtained from direct microscopy. The histological alterations were nonspecific and of mild intensity.

#### RESUMO

##### *Biopsia per-oral do jejuno na esquistossomose mansoni humana*

O acometimento do intestino delgado, na esquistossomose mansoni, foi estudado em 100 pacientes por meio da biopsia jejunal per-oral. Treze achavam-se nos períodos iniciais da infecção, 43 apresentavam a forma intestinal e 44 a hepatoplênica. Em todos os casos foi feita a microscopia direta, esmagando o fragmento ou parte do mesmo entre lâmina e lamínula, para a pesquisa de elementos esquistossomóticos. Em 52 casos, uma parte do fragmento de mucosa jejunal foi enviado para exame histopatológico, dos quais apenas 35 foram considerados adequados para análise e classificação histológica. A microscopia de lupa, para análise e classificação das vilosidades intestinais, foi realizada em 74 casos e a biopsia retal, para estudo comparativo, em 92 pacientes.

A biopsia jejunal per-oral evidenciou elementos esquistossomóticos em 43 dos 100 pacientes, sendo positiva em 69,2% nos casos de fase aguda, em 25,6% nos que apresentavam a forma intestinal e em 52,3% nos de forma hepatoplênica.

A biopsia retal, por sua vez, foi positiva em 80,4% sendo em 90,9% nos casos de fase aguda, em 95,0% nos de forma intestinal e em 63,4% nos de forma hepatoplênica.

A análise histológica revelou alterações discretas e inespecíficas na maioria dos casos, não evidenciando nenhum elemento esquistossomótico.

As vilosidades intestinais, apreciadas à microscopia de lupa, não diferiram do padrão normalmente encontrado em indivíduos não esquistossomóticos.

Os dados obtidos evidenciam que a mucosa do jejuno proximal é freqüentemente parasitada nos períodos iniciais da infecção es-

quistossomótica e nos pacientes com forma hepatoesplênica, muito mais do que revelam os estudos simplesmente histológicos.

A biópsia jejunal per-oral, com microscopia direta, mostrou ser um método valioso para o estudo do comprometimento do intestino delgado na esquistossomose mansoni humana.

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