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COMPARATIVE STUDY OF LIVER LYSOSOMES IN THE HEPATO-INTESTINAL AND HEPATO-SPLENIC FORMS OF HUMAN SCHISTOSOMIASIS MANSONI

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SUMMARY

The Authors' purpose was to find eventual variations of the number of lysosomes in the liver cells of patients with schistosomiasis mansoni, in both hepato-intestinal and hepato-splenic forms. Eleven patients of each group were selected. Evidentiation of lysosomes in a fragment collected from the right lobe of the liver by needle biopsy, through detection of acid phosphatase with Gomori's method. Counts of lysosomes by means of a reticulated occular lens KPL 8 x — Zeiss. Results of functional tests and histopathological examinations of the liver did not show differences between the two groups, with regard to the liver cell. No evidence of structural disturbances of the liver cell. Since there no functional or histological changes of the liver cell that might differentiate the two groups of patients, the fundamental conclusion of this study is perfectly acceptable: there was no statistically significant difference in the average number of lysosomes in the liver cell of the hepato-splenic and hepato-intestinal forms of human schistosomiasis mansoni.

INTRODUCTION

In 1955, DE DUVE et al. [10], after a series of papers that had been in process since 1951, on the rat liver, identified in that organ cytoplasmatic particles rich in enzymes, among then acid phosphatase. They suggested the name lysosome for these particles, pointing out, from the beginning, their rich content in hydrolitic enzymes and the possibility of an intracellular digestive function. Acid hydrolases are present in a latent form in the lysosome; they are capable of becoming active when there are changes in the permeability of the membrane or of the structure of the lysosome. Several papers were published with regard to the concept, functions and importance of lysosomes [3, 4, 5, 6, 7, 8, 9, 13, 14, 15, 26, 31, 32]. Among the enzymes detected in them, special attention should be drawn upon acid phosphatase, which can be identified by Gomori's method [19]. It is thus possible to recognize the morphology of these particles, hitherto known only by their biochemical expression. Several papers were carried out in human pathology, with special reference to those on hepathology [2, 12, 16, 17, 18, 21, 23, 24, 25, 29, 33]. But in a careful literature review we found no paper concerned with lysosomes of the liver cell in the hepato-intestinal and hepato-splenic forms of human schistosomiasis mansoni.

LITERATURE REVIEW

We found 3 papers concerned with acid phosphatase in experimental schistosomiasis

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mansoni, in mice. ANDRADE & BARKA [1], in 1962 observed mice infected with cercariae of *Schistosoma mansoni*, which were sacrificed in weekly intervals, from the second until the eighteenth week after infection. They verified that the acid phosphatase activity in liver cells was unchanged. EL-FIKY & BOLOS [11] in 1967 mentioned an increase of acid phosphatase in mice sacrificed 75 and 100 days after schistosoma infection. RAMADAN & MICHAEL [27], in 1969 observed infected mice sacrificed at intervals of 15 days until a maximum of 90 days. Using Gomori's method, they observed a nitid decrease of acid phosphatase activity at 65,75 and 90 days after infection.

MATERIAL AND METHODS

We selected 22 patients with schistosomiasis mansoni, all males between 19 and 38 years of age. Eleven presented the hepato-intestinal form (HI) and eleven the compensated hepato-splenic form (HE). Both groups presented hepatic lesion, with increased consistency of the liver and functional and/or histopathological disturbances. Palpable spleen in the hepato-splenic group. An important factor in the selection of these patients was absence of previous hepatopathy or extradigestive disease that might cause lesion of the liver.

Patients did not receive any treatment for schistosomiasis. The following complementary examinations were performed:

Feces, for search of viable eggs in the stools.

Evaluation of liver function: electrophoresis of plasma proteins (on paper), total and fractional bilirubinemia, glutamin-piruvic and glutamin-oxalacetic transaminases, BSP (bromosulphalein) retention (30 minutes of administration of BSP, 5 mg per Kilogram of body weight).

Needle biopsy of the liver through the 8th or 9th costal space, at right anterior axillary line, using a Vim-Silvermam needle. The

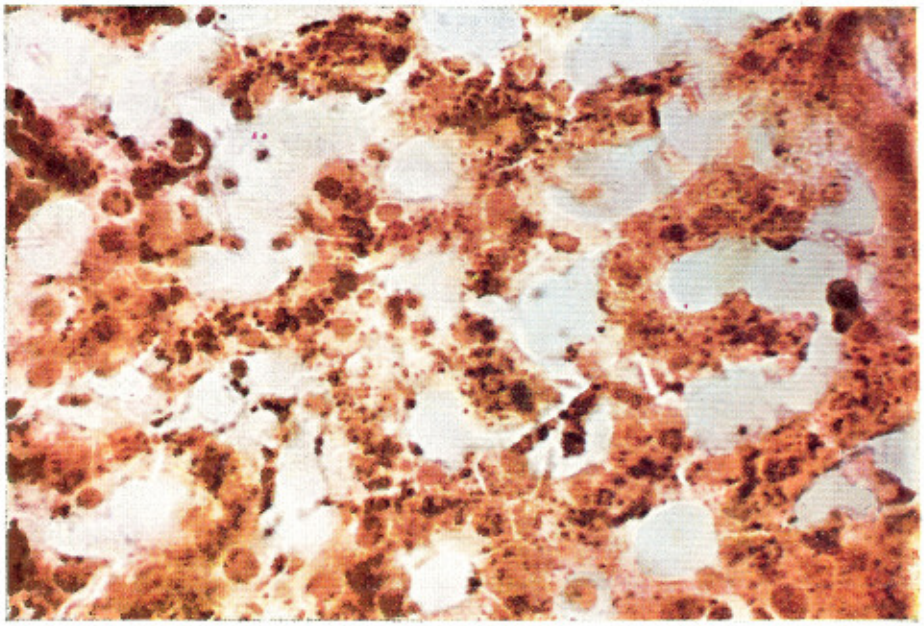

Fig. 1 — Photomicrography of a liver section showing distribution of lysosomes, of a patient with hepato-splenic schistosomiasis (case no. 15). Gomori's method for acid phosphatase. Zeiss photomicroscope. 580 ×

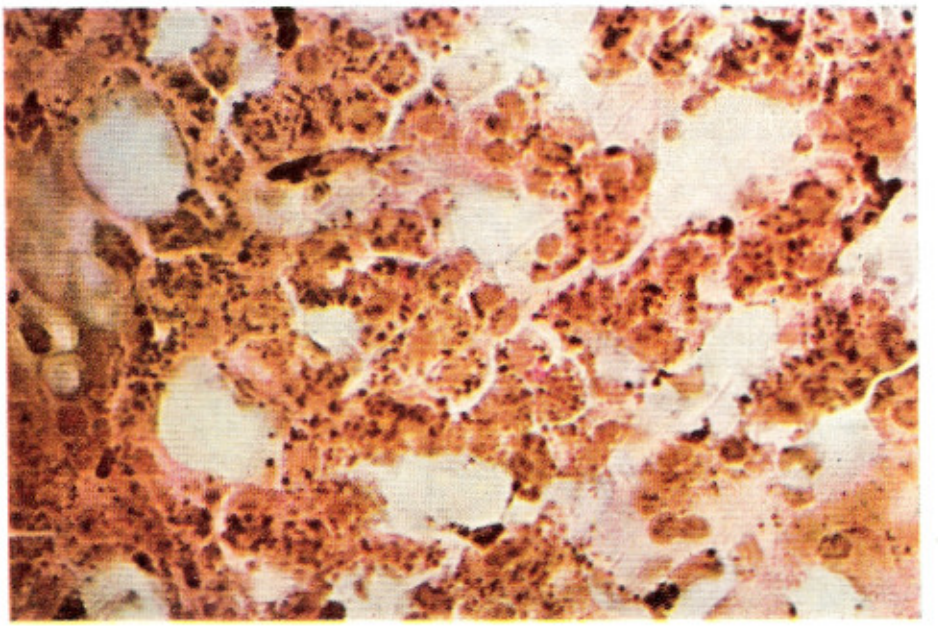

Fig. 2 — Photomicrography of a liver section showing distribution of lysosomes, of a patient with hepato-splenic schistosomiasis (case no. 21). Gomori's method for acid phosphatase. Zeiss photomicroscope. 580 ×

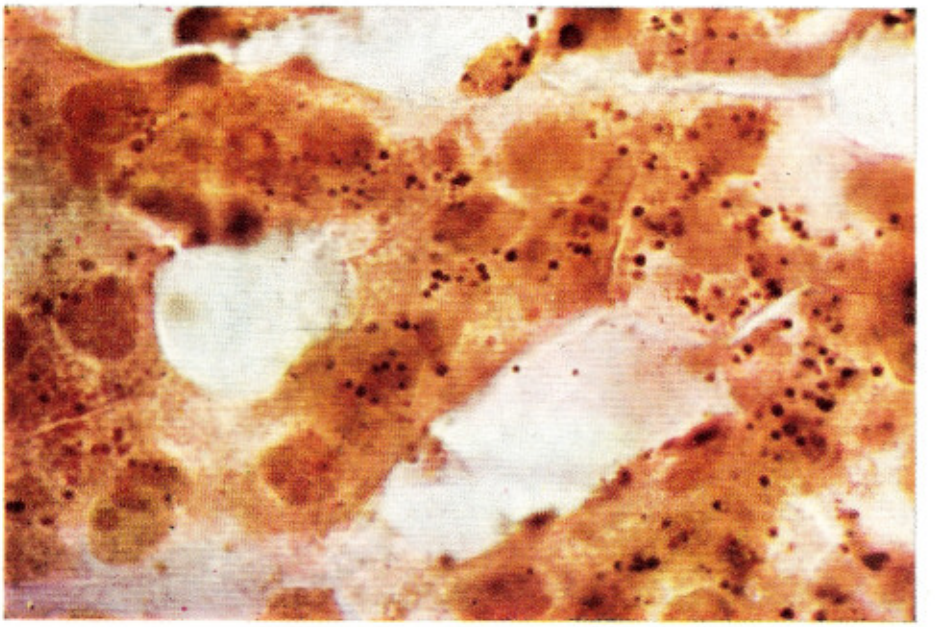

Fig. 3 — Photomicrography of a liver section; case no. 15. The material was photographed with the immersion objective used for our counts. Note that lysosomes can be identified clearly. Gomori's method for acid phosphatase. Zeiss photomicroscope. 1370 ×

material obtained was divided in two equal parts, so that histopathological and histochemical examinations corresponded to aspects of the same region of the right lobe of the liver.

Histopathological examination: specimens were fixed in formol 10% and stained with Hematoxylin-Eosin.

Evidentiation of lysosomes: Gomori's method [19] was used, which detects acid phosphatase in the lysosomes (Figs. 1, 2, 3).

Method used for lysosome counts: for a better evaluation of the number of lysosomes, a reticulated lens KPL8 x — Zeiss was used, usually employed to count reduced silver grains (histoautoradiography). An immersion objective 100 x was used (Fig. 3). Twenty squares of the reticulum were counted in each field of the slide, with the following distribution:

upper right angle	4 squares
upper left angle	4 squares
lower right angle	4 squares
lower left angle	4 squares
Center of the reticulum	4 squares
Total	20 squares

In each slide, ten fields were counted.

Statistical method: once the magnitude of the sampling was approved by SILVA LEME's [30] formula, the results were submitted to a contrast test between average rates (test "t" at level of 5%) according to the formula proposed by JOHNSON & LEONE [22].

RESULTS

In Table I, data regarding functional evaluation of the liver. In Tables II and

TABLE I

Summary of the results of liver function in the hepato-intestinal and hepato-splenic groups

Examination	Values found	HI		HE	
		no. of cases	%	no. of cases	%
BSP	normal (retention until 10%)	9	81.8	9	81.8
	above 10%	2	18.2	2	18.2
Albumin in absolute values	normal (3.40 — 4.30 g%)	4	36.4	4	36.4
	between 3.0 and 3.40 g%	5	45.4	3	27.2
	below 3.0%	2	18.2	4	36.4
Albumin in relative values	normal (50 — 58%)	0	0	0	0
	below normal	11	100.0	11	100.0
Gamablobulin in absolute values	normal (1.10 — 1.80 g%)	2	18.2	1	9.0
	above normal	9	81.8	10	91.0
Gamaglobulin in relative values	normal (14 — 22%)	0	0	0	0
	above normal	11	100.0	11	100.0

Obs.: HI = Hepato-intestinal schistosomiasis
HE = Hepato-splenic schistosomiasis
BSP = Bromosulphalein

TABLE II

Results of histopathological examination of the liver of patients belonging to the hepato-intestinal group

Case	H N	Ne.	Fib.	Gran.	P. I.	Egg	Conclusion of the histopathological examination
1		0	+	+	+	0	Compatible with hepatic schistosomiasis
2		0	+	+	+	+	Hepatic schistosomiasis
3		0	+	+	+	+	Hepatic schistosomiasis type Symmers
4		0	0	+	0	+	Hepatic schistosomiasis
5	H N	0	0	0	0	0	Liver histologically normal
6	H N	0	0	0	+	0	Liver histologically normal
7		0	0	+	+	0	Compatible with hepatic schistosomiasis
8		0	+	0	0	0	Moderate unspecific hepatic fibrosis
9		0	0	+	+	0	Compatible with hepatic schistosomiasis
10	H N	0	0	0	0	0	Liver histologically normal
11		0	+	+	+	+	Hepatic schistosomiasis type Symmers

Obs.: H N — Histologically normal
Gran. — Granuloma
Fib. — Fibrosis
Ne. — Necrosis
P. I. — Portal inflammation
0 — Absent
+ — Present

III, results of histopathological examinations of patients belonging to the hepato-intestinal and hepato-splenic forms. In Table IV, results of lysosome counts in both groups. For these counts, we chose slides incubated between 30 and 90 minutes, that showed the best staining. The sum of lysosomes counted in the 200 squares of each slide, allowed the evaluation of the same in each case studied. This count of lysosomes existent in each liver fragment allowed to establish a more reliable and uniform criterion, as well as a comparative study of the two groups studied.

The number of lysosomes counted varied between 84 and 328, with an average of 209.7 in the hepato-intestinal group, and between 107 and 450, with an average of 263.7 in the hepato-splenic group. In Table IV, statistical results show that there were no significant differences, at a level of 5%, between them.

We observed further, that the variability of the number of lysosomes was higher among

TABLE III

Results of histopathological examination of the liver of patients belonging to the hepato-splenic group

Case	H N	Ne.	Fib.	Gran.	P. I.	Egg	Conclusion of the histopathological examination
12		0	+	0	+	0	Liver fibrosis. Compatible with hepatic schistosomiasis
13		0	+	0	0	0	Sugestive of hepatic schistosomiasis
14		0	+	+	0	0	Hepatic schistosomiasis
15		0	+	0	+	0	Compatible with chronic hepatitis
16		0	+	+	+	0	Compatible with hepatic schistosomiasis
17		0	+	+	+	+	Hepatic schistosomiasis
18		0	+	+	+	0	Compatible with hepatic schistosomiasis
19	H N	0	0	0	0	0	Liver histologically normal
20		0	+	+	+	+	Hepatic schistosomiasis
21	H N	0	0	0	0	0	Liver histologically normal
22		0	+	+	0	0	Compatible with hepatic schistosomiasis

Obs.: H N — Histologically normal
Gran. — Granuloma
Fib. — Fibrosis
Ne. — Necrosis
P. I. — Portal inflammation
0 — Absent
\+ — Present

patients of the hepato-intestinal group (compare Pearson's variation coefficient). We also tried to relate the number of lysosomes found, with the functional and histopathological condition of the liver. The results show that there was no statistically significant differences in the average number of lysosomes in patients with normal albuminemia and bromosulphalein and in those with abnormal values in these tests (Table V).

The average number of lysosomes of patients with a histologically normal liver was statistically similar to those presenting a defined lesion of the liver (Table VI).

DISCUSSION

The tests to evaluate the function of the liver cell and the histopathological examination were only complementary to the main purpose of this paper; therefore, we comment their results briefly, without discussion.

Prothrombine activity was verified only to evaluate the condition for liver biopsy. According to ROSA BORGES [28], prothrombine activity may be falsely decreased in patients with hepato-splenic schistosomiasis, due to a disseminated intravascular coagulation and

TABLE IV

Results of lysosome counts in the hepato-intestinal and hepato-splenic groups

11 cases	no. of lysosomes	no. of lysosomes	11 cases
Cases no.	HI	HE	Cases no.
1	308	182	12
2	216	346	13
3	243	259	14
4	298	243	15
5	129	276	16
6	190	292	17
7	328	430	18
8	155	339	19
9	153	183	20
10	84	167	21
11	203	184	22
Average	209.7	263.7	
S	78.46	83.82	

Coefficient of variation (Pearson) HE — 31.78%
HI — 37.41%

Obs.: HI — Hepato-intestinal schistosomiasis
HE — Hepato-splenic schistosomiasis

TABLE V

Statistical correlation between the average number of lysosomes and the functional condition of the liver cell in the hepato-intestinal and hepato-splenic groups

Examination	with normal values	with abnormal values	Conclusion
	average no. of lysosomes		
Bromosulphalein	239.5	224.2	similar
Albuminemia	246.2	231.2	similar

TABLE VI

Statistical correlation between the average number of lysosomes and the histopathological condition of the liver in the hepato-intestinal and hepato-splenic groups

Histopathological examination	Average no. of lysosomes	Conclusion
Histologically normal	182	
Hepatic fibrosis	155	similar
Chronic hepatitis	243	similar
Compatible with hepatic schistosomiasis	276	similar
Hepatic schistosomiasis	250	similar
Type Symmers schistosomiasis	223	similar

consequent excessive consume of coagulation factors.

The Author asserts that in these patients prothrombine activity may be used as liver function test and evaluated as such, provided that consume coagulopathy is excluded.

Comparing the two clinical forms of schistosomiasis mansoni, we observed that the needle biopsy disclosed in all patients a qualitative similar histopathologic condition; quantitatively the changes were more pronounced in the hepato-splenic group. With regard to the liver cell, our results did not allow to establish any difference between the two groups, due to the absence of structural lesions, especially necrosis.

As to the liver cell function, our results showed no disturbances that would allow to differentiate the two groups. Our results agree, in their main points, with the Authors that studied patients with compensated hepato-splenic and hepato-intestinal forms of schistosomiasis mansoni. Although it is to some extent justifiable to assume that patients with hepato-splenic schistosomiasis have a

more serious disease, this fact cannot be accepted without restrictions. It is known that, in compensated patients there is lesser possibility of liver cell injury, secondary to the portal hypertension regimen. However, in spite of these known aspects, we had no idea of the behaviour of lysosomes, in these two more frequent forms of the disease; this justifies our interest in this research, with a comparative study of both groups.

Our basic considerations with regard to lysosomes were:

In the literature we found experimental papers, reporting mice liver cells infected with cercariae of *Schistosoma mansoni*, with unchanged [1], increased [11] or decreased [27] acid phosphatase activity. Our results, in the human liver, presented a statistically similar average number of lysosomes in the liver cell of both groups. Our results with regard to patients with normal liver function tests or histopathological examination, were statistically similar to those who had defined functional or histopathological disturbances (Tables V and VI). The interpretation of our results became more difficult due to the want of papers classifying the lysosome in the human schistosomiasis liver. The papers on animals are few and contradictory [1, 11, 27]. Furthermore, our papers represents the only attempt ever made to evaluate quantitatively the lysosomes population on a liver fragment of schistosomiasis patients, obtained by needle biopsy of the liver. Since there were no functional or histopathological changes of the liver cell that might differentiate the two groups studied, it is perfectly acceptable that no significant differences were found between the average number of lysosomes in the hepato-intestinal and hepato-splenic forms of schistosomiasis mansoni.

CONCLUSIONS

1) We found no statistically significant difference in the average number of lysosomes in the histologically normal liver and in the organ presenting structural changes.

2) We found no statistically significant difference between the average number of lysosomes and functional disturbances of the liver detected by bromosulphalein and albuminemia.

3) There was no statistically significant difference in the average number of lysosomes of the liver cell in both hepato-intestinal and hepato-splenic forms of human schistosomiasis mansoni.

RESUMO

Estudo comparativo dos lisossomos do hepatócito nas formas hépato-intestinal e hépato-esplênica da esquistossomose mansoni humana

O trabalho teve por objetivo a pesquisa de possíveis variações do número de lisossomos nos hepatócitos de doentes portadores de esquistossomose mansoni, formas clínicas hépato-intestinal e hépato-esplênica. Foram selecionados 11 doentes do grupo hépato-intestinal e 11 do hépato-esplênico compensado sem qualquer hepatopatia anterior e não tratados.

Evidenciação dos lisossomos, em fragmento colhido do lobo direito do fígado por punção-biópsia, através da detecção da fosfatase ácida existentes no seu interior, com o método de Gomori. Contagem dos lisossomos por intermédio de uma lente ocular quadriculada KPL 8 x — Zeiss.

Relativamente ao hepatócito, as provas de função e o exame histopatológico do fígado, mostraram resultados que não permitiram diferenciar os dois grupos. Ausência de alterações estruturais do hepatócito. Não havendo alterações funcionais ou histológicas do hepatócito que pudessem diferenciar os dois grupos de doentes é perfeitamente aceitável a conclusão fundamental deste trabalho: não houve diferença estatisticamente significante no número médio de lisossomos de hepatócito quando comparamos os achados nas formas hépato-intestinal e hépato-esplênico de Esquistossomose mansoni humana.

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