



Article/Artigo

Evaluation of the histopathological hepatic lesions and opportunistic agents in Brazilian HIV patients

Avaliação das alterações histológicas hepáticas e infecções oportunistas em pacientes brasileiros infectados pelo HIV

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ABSTRACT

Introduction: to evaluate the type of histopathological hepatic lesions and opportunistic agents in Brazilian HIV-infected patients. **Methods:** we examined 52 percutaneous liver biopsies of 50 HIV-infected patients who had at least two of the following conditions: fever of unknown origin, unexplained severe emaciation, hepatomegaly or abnormal liver chemistry. The specimens were cultured for mycobacteria and fungi and stained by standard procedures. **Results:** reactive patterns, granulomatous hepatitis and chronic active hepatitis were verified in 28 (54%), 11 (21%) and 8 (15%) of the patients respectively. Opportunistic infections were diagnosed in 18 (36%) patients: mycobacteria in 12 (24%), *Cryptococcus neoformans* in 5 (10%) patients and mycobacteria and yeast were isolated from the same liver fragment in one patient. **Conclusions:** mycobacteriosis was the most common opportunistic infection and liver tissue culture is an important method to detect opportunistic agents, even in the absence of histological lesions.

Key-words: Liver biopsy. Mycobacteriosis. AIDS. Liver histology.

RESUMO

Introdução: avaliar os tipos de lesões histopatológicas e infecções oportunistas de brasileiros infectados pelo HIV. **Métodos:** Foram analisadas 52 biópsias hepáticas percutâneas de 50 pacientes que apresentavam pelo menos duas das alterações: febre de origem indeterminada, emagrecimento inexplicado, hepatomegalia ou anormalidades na bioquímica hepática. O fragmento de tecido hepático foi submetido a histopatologia por métodos habituais e cultura para micobactéria e fungo. **Resultados:** padrão reacional, hepatite granulomatosa e hepatite crônica ativa foram encontrados em 28 (54%), 11 (21%) e 8 (15%) dos pacientes respectivamente. Infecções oportunistas foram diagnosticadas em 18 (36%) dos pacientes: micobactéria em 12 (24%), *Cryptococcus neoformans* em 5 (10%) pacientes e micobactéria e fungo foram isolados no mesmo fragmento em um paciente. **Conclusões:** micobacteriose foi a infecção oportunista mais comum e a cultura de tecido hepático foi um importante método para detecção de infecções, mesmo na ausência de lesões histológicas.

Palavras-chaves: Biópsia hepática. Micobacteriose. SIDA. Histologia hepática.

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INTRODUCTION

Research involving AIDS patients has determined a high prevalence of underlying hepatic abnormalities. The spectrum and prevalence of different diseases among patients with AIDS varies between countries¹.

Hepatic manifestations can be a harbinger of disseminated opportunistic infections in AIDS patients². Liver biopsy is a useful technique for the diagnosis of fever of unknown origin (FUO) in HIV-infected patients. Early biopsy should be considered in patients with hepatosplenomegaly and above-normal alkaline phosphatase levels³. Many liver and pancreatic lesions caused by opportunistic agents have been diagnosed based on autopsy results⁴.

Studies in underdeveloped countries suggest that hepatic tuberculosis is the most common liver disease in AIDS patients¹, followed by *Mycobacterium avium* complex infection, cytomegalovirus, toxoplasmosis, fungal infections, malignant tumors⁵ and concomitant pathologies².

Few studies of liver complications in Brazilian patients have been conducted, some involving case reports of hepatic pathology secondary to opportunistic diseases in HIV-infected patients, associated with tuberculosis⁶, histoplasmosis⁷, and *Salmonella-Schistosoma mansoni*⁸. Histopathological exams of liver biopsies were not performed in these Brazilian AIDS studies.

We examined hepatic histopathological findings and the diagnosis of opportunistic agents in Brazilian HIV patients who were not yet on antiretroviral drug therapy. This situation is possible in countries without resources and in patients who have not been diagnosed or placed on HIV therapy.

METHODS

The State Public Servants' Hospital (*Hospital Servidor Público Estadual*) is a public, tertiary care, teaching hospital that belongs to the Brazilian National Healthcare System (SUS). We analyzed 50 cases in which 52 percutaneous liver biopsy (PLB) had been performed. In two patients, the procedure was repeated due to clinical indications. Patients that presented at least two clinical or laboratory alterations including: prolonged fever, accentuated weight loss, hepatomegaly and liver function test abnormalities were selected for PLB. The following data was recorded for these patients: age, sex, alcohol abuse, use of medicines, as well as clinical symptoms, including fever, weight loss, hepatomegaly, signs of hepatic deficiency and abnormal laboratory parameters. Liver function test abnormalities were defined as above normal levels of serum transaminases, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), alkaline phosphatase (Aph) and glutamic-transferase (Gt). The normal values considered were: ALT 5-35U/l, AST 8-40U/l, Aph 30-40U/l and Gt up to 30U/l.

All percutaneous liver biopsies were performed with a Menghini needle, using standard techniques.

Unfixed tissue was cultivated for *Mycobacterium* and fungi. Formalin-fixed tissue sections were stained with hematoxylin and eosin, Masson's trichrome, Prussian blue, Gomori-methenamine silver, periodic acid-Schiff and Ziehl-Neelsen stains. Histology was analyzed by a single pathologist (DCCY). The Chi square and Fisher exact tests were used to compare categorical variables ($\alpha = 0.05$).

RESULTS

Among the 50 patients, 48 were male; median age was 35 years of age (range 16-60 years-old). Nine (18%) patients had alcohol abuse problems and 30% were illicit drug users.

Thirty (60%) patients presented opportunist infections, five (10%) had Kaposi's sarcoma, two (4%) had Hodgkin's lymphoma and one (2%) had idiopathic thrombocytopenic purpura. The clinical characteristics, laboratorial and histological findings of the patients are described in **Table 1**.

Liver biopsy showed histological alterations in 98% of the patients; the only patient who apparently had a normal liver (2%) was infected with mycobacteria. Two patients were submitted to two biopsies each, once after the initial diagnosis of acute hepatitis and the other 10 months later, after the disease had evolved to chronic active hepatitis in one patient and two months later in a patient with nonspecific reactive hepatitis that had evolved to acute cholestatic hepatitis.

Nonspecific reactive hepatitis (RH) involved hyperplasia and hypertrophy of the Kupffer cells (100%), hepatocellular degeneration (76%), inflammatory infiltration in the portal and periportal area (70%) and lobular (42%), sinusoidal dilatation (60%) and focal steatosis (56%) were observed in the liver biopsies. Granulomatous hepatitis (GH) presented lobular and portal granulomas, rarely formed by Langhans' cells, with minimal central necrosis. Mycobacteria were verified in three patients and yeast in five patients with RH. Among the 11 patients with GH, nine presented opportunistic infections (eight mycobacteria and one yeast, **Figure 1**). In one patient, extensive portal inflammation with Hodgkin's lymphoma was verified, associated with granulomas, with no infectious agents.

TABLE 1 - Description of the clinical characteristics, laboratory test results and histological findings of Brazilian HIV patients with liver problems.

Characteristics	Patients		Mean (range)
	n	%	
Clinical			
fever	26	52.0	
weight loss	35	70.0	
hepatomegaly	33	66.0	
splenomegaly	17	34.0	
Laboratory findings			
alanine aminotransferase (ALT)	33	66.0	114 (36-700)
aspartate aminotransferase (AST)	32	65.0	129 (51-791)
alkaline phosphatase (Aph)	23	50.0	277 (46-1620)
glutamic-transferase (Gt)	44	94.0	264 (31-1845)
Histological findings			
non-specific reactive hepatitis (RH)	28	54.0	
granulomatous hepatitis (GH)	11	21.0	
chronic active hepatitis (CAH)	8	15.0	
acute hepatitis (AcH)	2	4.0	
alcoholic hepatitis (AlH)	2	2.0	
normal liver (NL)	1	2.0	
Opportunistic liver infection findings			
tuberculosis	12	24.0	
cryptococcus	5	10.0	
tuberculosis and cryptococcus	1	2.0	

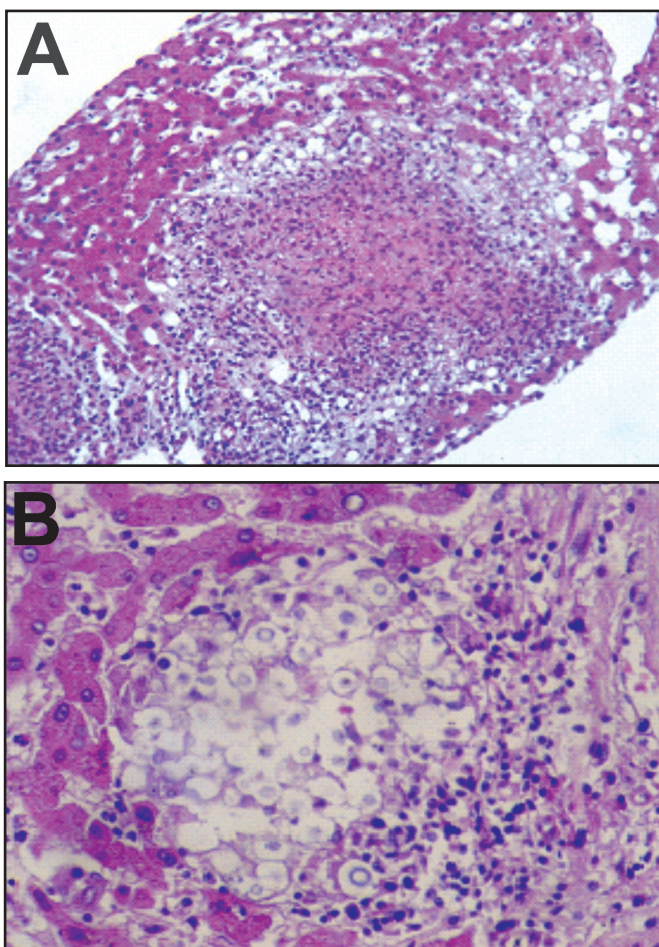


Figure 1 - A: Granulomatous hepatitis of tuberculosis (HE stain x 100); B. Cryptococcus neoformans in a granuloma in the liver parenchyma (HE stain x 400).

Mycobacteria were observed in 13 liver biopsies and yeast in six (five *Cryptococcus neoformans* and one unidentified yeast). One patient presented *Cryptococcus* and Mycobacteria in the same liver fragment. Liver biopsy permitted the diagnosis of opportunistic infections in nine (18%) patients, these included eight with mycobacteriosis and one with *Cryptococcus* infection.

The clinical alterations were not significantly correlated with the presence of opportunistic infections. Alkaline phosphatase was significantly altered in liver granuloma tissue. Levels of other liver enzymes, such as AST, ALT and Gt, were not correlated with histological findings. There was a significant relation between GH and opportunistic infections, specifically those caused by Mycobacteria. There was no correlation between histological findings and fungus infection (Table 2).

Among the 13 patients with a diagnosis of Mycobacteria, Ziehl-Neelsen staining was positive in six out of 12 (50%) patients, cultures were positive in all nine patients tested; *Mycobacterium tuberculosis* was identified in four of these. Due to a lack of hepatic tissue, histopathology was not performed for one patient and no cultures were made from samples of four patients. Among the patients diagnosed with fungi, two out of six were determined by staining and four out of five by specific culture.

TABLE 2- Correlation of clinical, laboratory and opportunistic findings with histological findings.

Clinical/ laboratory findings	RH		GH		CAH		Normal		p
	n	%	n	%	n	%	n	%	
Fever	15	54	8	73	—	—	—	—	0.27
Hepatomegaly	8	73	17	61	—	—	—	—	0.51
Weight loss	7	64	19	68	—	—	—	—	0.8
AST	16	57	8	73	6	75	—	—	0.5
ALT	17	61	6	55	4	50	—	—	0.8
Gt	24	86	10	100	5	71	—	—	0.22
Aph	11	27	9	90	2	29	—	—	0.013
Mycobacteria	3	11	8	73	1	12.5	1	—	0<0.01
Fungi	5	18	1	9	0	0	0	—	0.12
Opportunistic infections	8	28.5	9	82	1	12.5	—	—	0<0.01

RH: non-specific reactive hepatitis, GH: granulomatous hepatitis, CAH: chronic active hepatitis, AST: aspartate aminotransferase, ALT: alanine aminotransferase, Gt: glutamic-transferase, Aph: alkaline phosphatase.

DISCUSSION

Hepatic histological abnormalities were observed in all the patients; this frequency was higher than reported in other studies. Lanjewar *et al*¹ reported 58% patients with significant pathological lesions; the most common pathological processes involving the liver appeared to be secondary to infections¹. These findings could be explained by the phase of illness in these patients, with opportunistic disease occurring in 76%, along with drug abuse and frequent use of medicines.

Opportunistic infections were diagnosed in 36% of the patients, half of these by liver biopsy. Altered alkaline phosphatase levels were related to granulomatous hepatitis and mycobacteria infection. Garcia-Ordóñez *et al*³ studied 58 HIV-infected patients who underwent PLB for evaluation of FUO in Spain. The diagnosis was established in 51 (87.9%) patients; tuberculosis (50%) and leishmaniasis (20.7%) were the most common. PLB was diagnostic in 25 (43.1%) cases, helpful in 13 (22.4%), and normal or nonspecific in the remaining 20 (34.5%); they concluded that PLB is a useful technique for the diagnosis of FUO in HIV-infected persons and that

early PLB should be considered in patients with hepatosplenomegaly and increased alkaline phosphatase levels³. Other studies have reported similar conclusions^{2,9}.

Echejoh *et al*¹⁰ evaluated *postmortem* hepatic histopathological findings in HIV patients in Nigeria; most (65%) patients had clinical tuberculosis. Granulomatous hepatitis, chronic hepatitis, nonspecific reactive hepatitis and steatosis were the most common hepatic histopathological lesions, occurring in 34, 20, 15 and 12% of patients, respectively. Seven percent had normal histological features¹⁰.

In our study, the histopathological findings were often nonspecific. A correlation was determined between granulomatous hepatitis and opportunistic hepatic infections, with prevalence of *Mycobacterium tuberculosis*, and *Cryptococcus neoformans* among the fungi. Culture for mycobacteriosis and of the deep mycoses was an important approach for the detection of opportunists, even when Ziehl-Neelsen and PAS staining of the liver was negative.

In conclusion, Mycobacteriosis was the most prevalent hepatic infection in Brazilian patients and culture of liver fragments is important for the diagnosis of opportunistic infection.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

- Lanjewar DN, Rao RJ, Kulkarni SB, Hira SK. Hepatic pathology in AIDS: a pathological study from Mumbai, India. *HIV Medicine* 2004;5:253-257.
- Chang YG, Chen PJ, Hung CC, Chen MY, Lai MY, Chen DS. Opportunistic hepatic infections in AIDS patients with fever of unknown origin. *Journal of the Formosan Medical Association* 1999;98:5-10.
- Garcia-Ordóñez MA, Colmenero JD, Jimenez-Onate F, Martos F, Martínez J, Juárez C. Diagnostic usefulness of percutaneous liver biopsy in HIV-infected patients with fever of unknown origin. *Journal of Infection* 1999;38:94-98.
- Danesi G, Pianta P, Mastroianni A, Cicognani C, Cristoni L, Sama C. Hepatic and pancreatic disease in patients with acquired immunodeficiency syndrome (AIDS). *Minerva Medica* 1999;90:123-131.
- Shakhgildian VL, Kravchenko AV, Parkhomenko Iu G, Tishkevich OA, Serova VV, Gruzdev B M. Liver involvement in secondary infections in HIV-infected patients. *Terapevticheski Arkhiv* 2002;74:40-43.
- Barone B, Kreuzig PL, Gusmão PM, Chamie D, Bezerra S, Pinheiro P, et al. Case report of lymph nodal, hepatic and splenic tuberculosis in an HIV-positive patient. *Brazilian Journal Infectious Disease* 2006;10:149-153.
- Borges AS, Ferreira MS, Silvestre MT, Nishioka SA, Rocha A. Histoplasmosis in immunodepressed patients: study of 18 cases seen in Uberlândia, MG. *Revista da Sociedade Brasileira de Medicina Tropical* 1997;30:119-124.
- Lambertucci JR, Rayes AA, Gerspacher-Lara R. *Salmonella-S. mansoni* association in patients with acquired immunodeficiency syndrome. *Revista do Instituto de Medicina Tropical de São Paulo* 1998;40:233-235.
- Piratvisuth T, Siripaitoon P, Sriplug H, Ovarlarnporn B. Findings and benefit of liver biopsies in 46 patients infected with human immunodeficiency virus. *Journal of Gastroenterology and Hepatology* 1999;14:146-149.
- Echejoh GO, Mandong BM, Tanko MN, Manasseh AN, Okeke EN, Agaba E I. Hepatic histopathological findings in HIV patients at postmortem in Jos university teaching hospital, Nigeria. *Tropical Doctor* 2006;36:228-231.