OPPORTUNISTIC INFECTIONS IN THE LIVER OF HIV-INFECTED PATIENTS IN THAILAND: A NECROPSY STUDY

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Abstract. Liver necropsy from patients infected with human immunodeficiency virus was analyzed in 117 cases. Wide ranges of opportunistic infections were recorded in 47%. Cryptococcosis (21.4%) was the most outstanding infection, followed by tuberculosis (16.2%), cytomegalovirus (5.1%) and penicillosis (3.4%). Non-specific alterations of the liver tissues included fatty steatosis (49.6%), fibrosis (55.6%), portal inflammation and reactive hepatitis. Cases of chronic active and chronic passive hepatitis and one case of hepatocellular carcinoma were reported. In the infected liver, predominant pathological changes included granuloma and spotty necrosis, which were attributed to tuberculous hepatitis. Infection with *Cryptococcus* usually showed no associated pathological change. The sensitivity for the clinical diagnosis of *Cryptococcus* was 88.8% and specificity was 91.7%. For tuberculosis, sensitivity was 20% and specificity was 67.9%.

INTRODUCTION

The study of opportunistic infections in patients infected with human immunodeficiency virus (HIV) has an important impact in health and medicine. Since the emergence of the fatal disease, acquired immunodeficiency syndrome (AIDS), ranges of opportunistic infections have been reported. It may result from viral, bacterial, protozoal or fungal diseases. Wilkins *et al* (1991) reported a study on 101 liver biopsies from human immunodeficiency virus infected patients in a six year period between 1984-1989 and detected the following opportunistic infections: cytomegalovirus, *Histoplasma encapsulatum*, *Pneumocystis carinii*, *Cryptococcus neoformans* and *Leishmania donovani*. Involvement of the liver with opportunistic infections has been recognized and usually reflects disseminated rather than primary disease (Bach *et al*, 1992). Still, there is only limited information on the prevalence and influence of HIV/AIDS and opportunistic coinfections, especially in the Southeast Asia where the infectious agents are different from Western. It is however, our attempt to study the liver pathology in HIV-infected patient with special emphasis on the opportunistic infections.

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MATERIALS AND METHODS

Inclusion criteria

1. Patients dying with HIV infection at Ramathibodi Hospital, Mahidol University, Pramongkutklao Hospital and the Hospital for Tropical Diseases, Mahidol University.

2. Males and females with anti-HIV screening tests positive, no age limit.

3. Written informed consent by the closest relative or by the patient himself on admission in agreement of any procedure performed on him by the physician or forensic cases, which require autopsy by law.

Procedure

Liver necropsy was performed using a disposable liver biopsy set. Sleeves of liver tissues, 0.2 cm in diameter of variable length was fixed in 10% buffered formalin for 24-48 hours. The tissues were then being processed in series of alcohol, acetone and melted paraffin; embedded in paraffin blocks and sectioned with a microtome to the thickness of 3-4 microns. Slices of tissues were laid in a dry and clean glass slide then stained with modified Hematoxylin and Eosin stain, a standard method for histopathological evaluation.

Pathological changes of the liver tissues were recorded. Special stains were employed to identify possible pathogens depending on the changes observed

with the routine modified hematoxylin and eosin stain. The stains include Kinyoun's stain, Taylor's stain, Periodic Acid Schiff's stain (PAS), Gomori Methenamine stain (GMS), Mucin stain and Masson Trichome stain. These are specific histologic studies for mycobacteria, other bacteria and fungi, respectively. Masson Trichome stain was used to identify early fibrosis in cases like chronic active hepatitis.

Data collection and analysis

Pertinent clinical data and pathological changes were recorded. Clinical data comprised of sex, age, diagnosis, enlargement of liver and pertinent laboratory liver profiles. Pathological changes included organisms detected and other associated liver damage.

For analysis, a descriptive study was employed. Sensitivity and specificity in clinical diagnosis were analyzed. Some data was too small for chi-square test at 95% confidence interval. Data were recorded using the dBase III program and analysed using Epi Info 6 program.

RESULTS

A total of 117 cases were included in this study. There were 27 females and 86 males. No data regarding sex was recorded in four cases. The ages ranged from 2-74 years old. Clinical data is tabulated in Table 1. The most common clinical presentation was neurological symptoms (24 of 48 cases, 50.0%). Patients usually presented with headache and alteration of consciousness. *Cryptococcus* and tuberculosis of the liver were detected in nine (37.5%) and five cases (20.8%), respectively, among patients with neurological symptoms as a disseminated process. The most common clinical diagnosis before death was tuberculosis and cryptococcosis. For clinical data related to liver, hepatomegaly was identified in 16 of 46 cases (34.8%). The means of pertinent liver profile were tabulated in Table 1. The means of all the laboratory data were compared with pathological diagnosis and no statistical significance at 95% confidence interval was found.

Pathological findings are tabulated in Tables 2 and 3. Liver histology was analysed in 117 cases infected with human immunodeficiency virus. Normal findings could only be documented in 45 patients (38.5%). In the majority of cases, non-specific alterations were seen such as fatty steatosis (49.6%), portal inflammation, and reactive hepatitis. Of the 58 cases of fatty steatosis, it could be classified into mild (36.8%), moderate (7.7%) and severe (5.1%). There is no statistical significance between fatty steatosis and different organisms detected (p=0.1133). Fibrosis was generally detected in 55.6% which was classified as mild (39.3%), moderate

Table 1
Clinical presentations of HIV-infected patients.

Sex		
Male	86	
Female	27	
No.	113	
Age	2-74	years old
No.	60	
Clinical symptoms		
Fever	3	
Respiratory symptoms	15	
Neurological symptoms	24	
GI symptoms	6	
No.	48	
Liver size[a]		
Not palpable	30	
1	5	
2	3	
3	5	
4	2	
5	1	
No.	46	
Laboratory data		
TB (mg/dl)	0.91	(n=38)
DB (mg/dl)	0.45	(n=38)
AP (U/l)	181.82	(n=38)
Chol (mg/dl)	170.30	(n=33)
SGOT (U/l)	98.34	(n=38)
SGPT (U/l)	57.03	(n=38)
TP (g/dl)	7.45	(n=31)
Alb (g/dl)	3.01	(n=38)
Trig (mg/dl)	149.92	(n=25)

[a]Liver size 1= one cm below right costal margin
2= two cm below right costal margin
3= three cm below right costal margin
4= four cm below right costal margin
5= five cm below right costal margin

Table 2
Opportunistic infections in liver tissues of HIV-infected patients (n=117).

Infection	n=117
Cryptococcosis	25 (21.4%)
Tuberculosis	19 (16.2%)
Cytomegalovirus	6 (5.1%)
Penicillosis	4 (3.4%)
Small fungus	1 (0.9%)

Table 3
Pathological changes related to the organisms of the liver in HIV-infected patients.

Diagnosis	Pathological changes					
	Spotty necrosis	Granuloma	Vague granuloma	Lymphocytes at lesion	No change	Total
Cryptococcosis	-	5 (20.0%)	1 (4.0%)	8 (32.0%)	11 (44.0%)	25 (100%)
Tuberculosis	6 (31.6%)	8 (42.1%)	3 (15.8%)	-	2 (10.5%)	19 (100%)
Cytomegalovirus	2 (33.3%)	-	-	-	4 (66.7%)	6 (100%)
Penicillosis	-	1 (25.0%)	1 (25.0%)	-	2 (50.0%)	4 (100%)
Small fungus	-	-	-	-	1 (100.0%)	1 (100%)

(12.0%) and severe (4.3%). There is no statistical difference between the degree of fibrosis and the presence of organisms (p=0.8036). Wide ranges of opportunistic infections were recorded in 47.0% (55 of 117). Infections with *Cryptococcus* was the most outstanding infection among others (21.4%), followed by tuberculosis (16.2%), cytomegalovirus (5.1%) and penicillosis (3.4%). At 95% confidence interval, there was statistical significance between the two leading organisms, *Cryptococcus* and tuberculosis and the associated pathological findings (p=0.00025). Chronic active and chronic passive hepatitis were also detected. One case of hepatocellular carcinoma was diagnosed in a young man.

Pathological changes related to *Cryptococcus* varied. Ordinary routine modified hematoxylin and eosin staining could readily see the organism by gently moving the fine adjustment knob of the microscope. This could help to visualize the refractivity of the organism's capsule (Fig 1). Severely infected liver tissues could easily be identified even without special stain with PAS, GMS or Mucin stain. Some cases were difficult to identify. Early lesions usually displayed minimal changes, others had no associated changes at all. Such cases required special stains. The changes observed were usually minimal lymphocytic infiltration around these capsular fungi. Granuloma with giant cells was also seen as associated finding. The organism caused the destruction of hepatocytes in a small spotty pattern.

Infection with *Mycobacterium* (16.2%) showed interesting associated pathologic changes. These included granulomas of different types. Majority of the cases (42.1%) exhibited changes observed in non-immune cases, which was the formation of granuloma with identifiable Langerhans' giant cells. Other groups consisted only of spotty necrosis (31.6%). (Fig 2) and vague granuloma. They displayed collections of histiocytes with minimal lymphocytic responses but no typical Langerhans' giant cells. It was observed that liver with well formed granuloma often displayed a minimal number of acid fast bacilli. But in cases of vague granuloma or spotty necrosis, numerous organisms could be visualized (Fig 3). Organisms could be detected in the hepatocytes, in the Kupffers cells, caseating necrotic areas and within the histiocytes.

Cytomegalovirus affected both hepatocytes (Fig 4) and endothelial cells (Fig 5). Identification of this virus needs meticulous observation. No inflammatory change was associated with this infection. They often showed minimal involvement in the liver.

Penicillosis was found in 4 cases (3.4%). Zero lymphocytic responses were observed. It infected the hepatocytes and caused destruction of hepatocytes in spotty pattern. The organism requires GMS stain to be identified (Figs 6, 7).

DISCUSSION

Cryptococcus was the most prevalent opportunistic organism detected in this study. It was not usually seen in previous reports (Beale *et al*, 1995; Santos *et al*, 1991, Jeena *et al*, 1996) or merely found in low percentage (Trojan *et al*, 1998). This finding should be worrying for clinicians who deal with HIV-infected patients, such that proper antifungal prophylaxis be instituted. Presence of the organism in the sinusoids suggested disseminated involvement of various organs.

For tuberculosis, the pathologic changes of vague granuloma and spotty necrosis could be misdiagnosed if there was no awareness of the changes in the immunocompromised patients. Such changes could be attributable to low cell mediated immunity in AIDS patients so that their immune re-

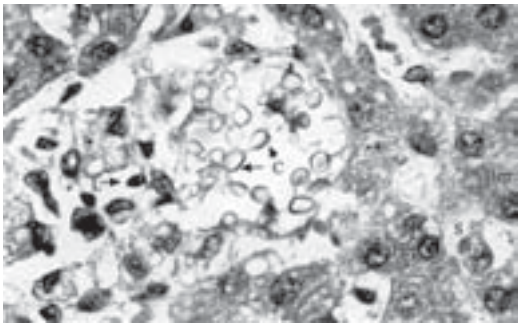

Fig 1–*Crytococcus neoformans* in the liver. Some shows buddings activities. Note the destruction of hepatocytes. Mucin stain, 400x. C=*Crytococcus neoformans*, Hep=hepatocytes, S=sinusoids, L= lymphocytes.

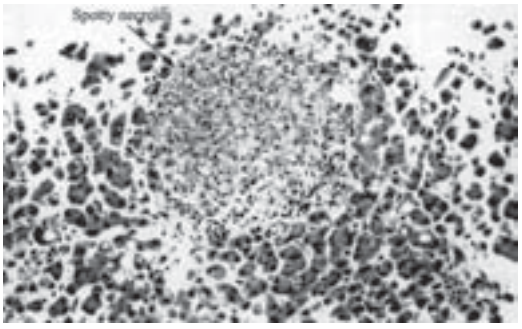

Fig 2–Spotty necrosis in the liver. Modified hematoxylin and eosin stain, 100x.

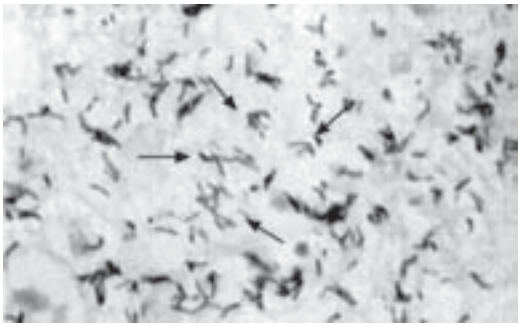

Fig 3–Positive acid fast bacilli in the necrotic area of the liver (→). Kinyoun's stain, 1,000x.

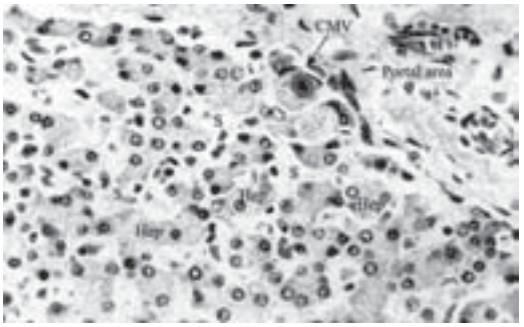

Fig 4–Cytomegalovirus (CMV) infecting hepatocyte. Modified hematoxylin and eosin stain, 400x. Hep=hepatocytes, S=sinusoids.

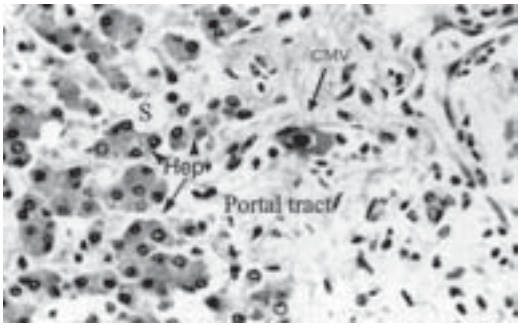

Fig 5–Cytomegalovirus infecting the endothelium of hepatic vein. Note the enlargement of the endothelium, the typical intranuclear inclusion and perinuclear halo. Modified hematoxylin and eosin stain, 400x. Hep=hepatocytes, S=sinusoids.

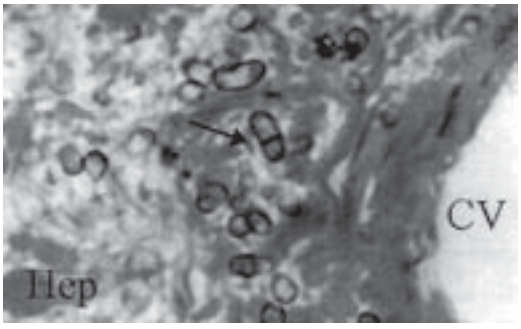

Fig 6–*Penicillium marneffei* in the liver. Note the central banding (→). Gomori Methenamine stain, 1,000x. Hep=hepatocytes, CV=central vein.

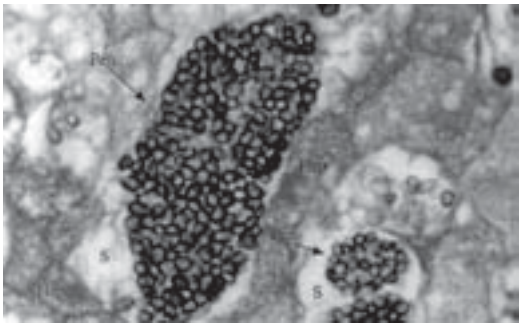

Fig 7–*Penicillium marneffei* packed within the sinusoids of the liver. Gomori Methenamine stain, 1,000x. Pen= *Penicillium marneffei*, Hep=hepatocytes, S=sinusoids.

sponses were unable to form a typical giant cells. With special stains like Kinyoun's stain or AFB stain, acid fast bacilli could easily be identified. Poles *et al* (1996) studied biopsy specimens in USA and found the most common biopsy-derived diagnosis to be *Mycobacterium avium complex* (MAC) noted in 17.4% of biopsies, while Kahn *et al* (1986) detected granuloma in 48% (10/21) and were most often a manifestation of MAC.

Cytomegalovirus was detected in 6 cases (5.1%). This finding was higher than previous reports (Cappell *et al*, 1990, Astagneau *et al*, 1990).

Penicillosis is an important disease of third world countries. It is rarely seen and often not detected antemortem if it involves the internal organs. It is differentiated from histoplasmosis by it's central banding pattern.

Among the non-infectious diseases, only one hepatocellular carcinoma was found in a young man, unlike previous reports by Astagneau *et al* (1990) where they found 19% of the liver tissues had Kaposi's sarcoma and two cases of hepatic lymphoma.

Accuracy of clinical diagnosis was compared with pathological diagnosis for the leading detected diseases. The sensitivity for the diagnosis of *Cryptococcus* was 88.8% and specificity was 91.7%. For tuberculosis, sensitivity was 20% and specificity was 67.9%. Due to small number of matched data, sensitivity and specificity for cytomegalovirus and *Penicillium* could not be evaluated.

There was no histologic feature in the liver as being specific for human immunodeficiency virus (HIV) infection. Pathologic changes in the liver tissues of HIV-infected patients can be attributed to the presence of opportunistic infections, where *Cryptococcus* had the highest prevalence in this study. This is usually part of a disseminated multi-organ process arising in immunocompromised patients. The use of drugs is another factor causing liver abnormalities.

AIDS is alarmingly increased, specially in Third World countries. Similar studies as a form of surveillance in particular region are recommended to constantly keep track of the changing opportunistic infections in HIV/AIDS. The results from this study, serve as basic information on the commonly occurring opportunistic infections in Thailand. This information could help physicians to be aware of infectious diseases prevalent in Thailand. Moreover, a precise choice of antibiotics, antifungal or antiviral agents can be correctly employed. Based on the outcome, liver biopsy may help early diagnosis of opportunistic infections in early HIV infection.

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