

# Liver Enlargement Associated with Opportunistic Infections in Patients with Human Immunodeficiency Virus Infection

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## Abstract

**Background & Aim.** Liver disease is commonly present in human immunodeficiency virus (HIV) infection. The aim was to determine the frequency of liver enlargement and its association with opportunistic infections in patients with HIV infection. **Patients and methods.** A total of 400 HIV-infected patients were investigated. Commercial kits (Ortho EIA; BioRad, ELISA) were used for detection of serum specific antibodies to HIV, hepatitis C virus, surface antigen of hepatitis B virus, and cytomegalovirus. Liver tissues were stained for various microorganisms. The electronic data base SPSS for Windows (version 10.0) was used for statistical analysis. A  $p < 0.05$  was considered significant. **Results.** Ultrasonographic screening revealed liver enlargement in 63.75% of HIV patients. In 40.7% the right lobe size varied from 140 mm to 160 mm. Of those with hepatomegaly, 60.7% had AIDS. Hepatitis C and B viruses and Mycobacterium tuberculosis were detected in 50%, 29% and 18% of patients, respectively. Histological changes were mostly non-specific. Liver pathology depended on the degree of cellular immune deficiency, particularly in patients with HBV co-infection. In a minority of patients (32.5%), the histology revealed granulomatous hepatitis. Liver function tests were abnormal in 46%. **Conclusion.** Liver enlargement is common in HIV-infected patients mostly in association with hepatitis C and B viruses and Mycobacterium tuberculosis.

## Key words

Human immunodeficiency virus – hepatomegaly – hepatitis C virus – hepatitis B virus – mycobacterium tuberculosis.

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## Introduction

The acquired immunodeficiency syndrome (AIDS) is the terminal stage of chronic infection caused by the human immunodeficiency virus (HIV) with ability to damage immune competent cells. Dysfunction and gradual elimination of CD4 positive T lymphocytes is the basic pathological process attributed to this infection. The infected host has diminished response, mostly to HIV and also to other infections that require competent cellular immunity for their control and elimination [1, 2].

Human immunodeficiency virus can involve all gastrointestinal organs including the hepatobiliary system [3, 4]. However, damage of the hepatobiliary system can be the result of HIV infection itself, the influence of opportunistic microbes or various toxic effects of drugs. Features of liver disease can be found in all stages of HIV infection. Previous investigations have shown one or more opportunistic infections of the liver approximately in 70% of patients with HIV [5, 6].

## Aim

The aim of this study was to determine the frequency of liver enlargement and its association with opportunistic infections in HIV-infected patients.

## Patients and methods

This retrospective study included 400 HIV-infected patients aged 16 to 65 yrs (mean age  $41 \pm 12$ ; males 288) consecutively hospitalized in the Institute for Infectious and Tropical Diseases in Belgrade, from January 1, 2002 to January 1, 2006. Complete medical history, routine clinical and diagnostic procedures including an ultrasound examination of the abdomen, and standard biochemical liver function tests (LFTs) were performed in all patients. The minority of patients took mono and/or dual antiretroviral therapy including nucleoside reverse transcriptase inhibitors, since the study period comprised pre-HAART era.

Detection of serum specific total antibodies to hepatitis C virus (HCV) and HIV (anti-HCV and anti-HIV)

confirmed by Western blot, of cytomegalovirus (CMV) and surface HBV antigen (HBsAg) were performed using commercial kits (Ortho EIA; BioRad, ELISA). Liver tissue was obtained by aspiration needle biopsy in all 255 patients with hepatomegaly and assessed with standard histological procedures. In addition, the samples were stained for microbiological investigations (i.e. Mycobacterium tuberculosis -MT, Mycobacterium avium-intracellulare - MAI, Cryptococcus neoformans, etc.), while CMV was identified observing typical inclusions or isolating the virus in fibroblast cell culture.

The electronic database organized in the Statistical Package for Social Science (SPSS) for Windows (version 10.0) was used for statistical analysis. The results are expressed as absolute numbers, mean  $\pm$  SD, and percentage. The level of significance was set at  $p < 0.05$ . Data are presented with 95% confidence intervals.

## Results

The number of patients with hepatomegaly was 255 (63.75%) among HIV-infected patients. There were 177 (69.4%) males. The majority of HIV-infected patients with hepatomegaly - 118 (46.3%) belonged to the age group of 20 to 40 years. Among patients with hepatomegaly, 155 (60.8%) had AIDS.

Enlargement of the liver right lobe was discovered on physical and ultrasound examination ranging from 10 mm to 60 mm higher than normal size (120 mm). Simultaneously, abnormal morphological features were seen in 68% of patients by ultrasound examination. Most frequently, the size of the liver right lobe was between 140 mm and 159 mm (40.7%).

Validity of standard laboratory parameters for diagnosis of liver impairment in HIV-infected patients is presented in Table I. Analysis of laboratory data showed that increased ALT activity was associated with liver damage in 46% of HIV patients confirming it as the best marker for detecting liver disease as compared to other enzymes.

Non-specific histological changes were most commonly seen (in proportions ranging from 88% to 11%) in liver tissues. They were expressed as one or several types of alterations in an individual sample. Their variety and dependence on the grade of impaired cellular immunity in the HIV-infected patients is presented in Table II.

**Table I.** The biochemical function liver tests in HIV-infected patients

Parameter	Normal value	Range	Mean $\pm$ SD	Association with hepatomegaly (%)
Total bilirubin	<17 $\mu$ mol/L	16-167	64 $\pm$ 52	18
ALT	<34 IU/L	40-870	441 $\pm$ 280	46
GGTP	<40 IU/L	44-189	86 $\pm$ 68	38
AP	<90IU/L	90-340	220 $\pm$ 110	22

ALT=Alanine aminotransferase; GGTP=Gamma glutamyltranspeptidase; AP=Alkaline phosphatase

**Table II.** Frequency of non-specific histological changes in liver tissues depending on the grade of cellular immunodeficiency in HIV-infected patients

Type of changes	CD4 T Ly count >200/mm <sup>3</sup> (%) (n=147 pts)	CD4 T Ly count <200/mm <sup>3</sup> (%) (n=108 pts)	p*
Hyperplasia and hypertrophy of Kupffer cells	37 (25%)	75 (70%)	<0.001
Dilatation of sinusoids	18 (12%)	69 (64%)	<0.001
Erythrophagocytosis	-	37 (34%)	-
Cholestasis	18 (12%)	12 (11%)	>0.05
Hepatocyte necrosis	117 (80%)	12 (11%)	<0.001
Inflammation	129 (88%)	34 (31%)	<0.001
Fibrosis	18(12%)	41 (38%)	<0.001
Fatty infiltration	91 (62%)	58 (54%)	>0.05

\*Fisher's exact test

Assessment of liver biopsies showed different non-specific histological alterations depending on the grade of the impairment of cellular immunity. The most frequent non-specific changes in liver tissue in patients with a CD4 T lymphocyte count higher than 200/mm<sup>3</sup> were inflammation, hepatocyte necrosis and fatty infiltration (steatosis). In patients with a CD4 T lymphocyte count lower than 200/mm<sup>3</sup>, hyperplasia and hypertrophy of Kupffer cells and dilatation of sinusoids was noticed in 70% and 64% of patients, respectively. These were more frequent than in patients with a higher CD4 T lymphocyte count. The frequency of fatty infiltration was not significantly different between patients with higher and lower CD4 count. Erythrophagocytosis was noticed only in patients with lower CD4 count, and fibrosis (38%) was more commonly seen in the same group.

Liver cirrhosis was found in HCV and HBV positive patients in 46/127 and 30/76 liver tissue specimens, respectively, without significant difference ( $p>0.05$ ).

Liver granulomas were found in 83 (32.5%) patients. Stained with specific procedures (e.g., Ziehl-Nielsen, Methenamin Silver, etc.), MT was discovered in 36 (43.4%) patients. In the majority of the patients, tuberculous granulomas were without caseous necrosis and giant multinuclear cells. Characteristic CMV inclusions were seen in 6 (7.2%) granulomas. In 13 (15.7%) granulomas no etiological cause was recognized.

Frequency and morphological changes in the liver associated with opportunistic infections in HIV patients and their dependence of developed AIDS in patients with hepatomegaly are presented in Table III.

Hepatitis C virus infection was serologically detected in 128 (50.2%) patients with liver enlargement. Hepatitis B virus and CMV were detected in 75 (29.4%) and 12 (4.7%) patients, respectively. Histological assessment indicated infection with these three viruses in 90% of patients. Features

**Table III.** Opportunistic infections associated with hepatomegaly in HIV-infected patients

Etiology <sup>1</sup>	Frequency (%)	Liver histological changes	AIDS pts (n=100)	non-AIDS pts (n=100)	p*
Hepatitis C virus	128 (50.2)	127 (92.2)	74	53	>0.05
Hepatitis B virus	76 (29.2)	76 (100)	34	42	<0.05
CMV	12 (4.7)	12 (100)	7	5	>0.05
MT	46 (18)	46 (100)	46	–	
MAI	8 (3.1)	8 (100)	8	–	
Cryptococcus neoformans	6 (2.3)	6 (100)	6	–	
Unknown	13 (5.1)	13 (100)	6	7	>0.05

<sup>1</sup>Serological and/or detection from the liver tissue; CMV=Cytomegalovirus; MT=Mycobacterium tuberculosis; MAI=Mycobacterium avium-intracellulare; \*Statistical significance in frequency between AIDS and non-AIDS patients (Fisher's exact test)

of chronic hepatitis B virus depended on the grade of the impairment of cellular immunity, i.e., patients with AIDS had mild chronic hepatitis while patients with less impairment of cellular immunity mostly had severe chronic hepatitis. In general, a variety of progressive histological features (activity and fibrosis) were found in chronic viral B and C infection, from mild chronic hepatitis to liver cirrhosis. Statistical analysis of AIDS and non-AIDS patients revealed equal distribution of HCV and CMV infection, while HBV infection was found more frequently in non-AIDS patients ( $p < 0.05$ ). Infections with Mycobacterium (tuberculosis and avium) as well with Cryptococcus neoformans were present exclusively in AIDS patients.

Data analysis showed that the most frequent bacterial infection was MT, detected in 46 (18%) patients. The most frequent infections in AIDS patients with enlarged liver were HCV and MT, while HCV and HBV were the most frequent viral infections detected in patients without AIDS. Simultaneous infection with HBV and HCV was present in 16 (10.3%) patients with AIDS. In 13 (5%) HIV-infected patients with liver enlargement, no opportunistic infections were detected.

## Discussion

Liver disease is common in HIV infection and can cause the death of patients even in those previously considered cured [3]. Therefore, physicians and patients should be aware of the symptoms, laboratory and visual methods for diagnosis, drug toxicities and available treatment of HIV-related liver conditions. Important causes of chronic liver disease in patients with HIV are co-infection with hepatitis B or C viruses and various bacteria and fungi as opportunistic pathogens, drug or alcohol abuse, insulin resistance or medication side-effects [7, 8]. Symptoms and signs, such as pain on the right side of the abdomen, hepatomegaly, jaundice, fever of unknown origin, fatigue, malaise, itching, and elevated LFTs require urgent and complete medical evaluation.

Our investigation showed a high frequency of hepatomegaly in HIV-infected patients (63.7%) on routine ultrasound examination. The majority of these patients had

AIDS as the terminal stage of this infection. In addition to liver enlargement, numerous morphological changes in the liver were seen in these patients. This finding confirmed that abdominal ultrasound examination is the crucial method not only for diagnosis of liver enlargement but also for following-up the progression of the disease [1, 2].

This study shows that standard laboratory parameters are important for diagnosis of liver function in HIV-infected patients. According to our data, liver impairment was found in 18-46% of patients depending on the used parameter. Activity of ALT was confirmed as the best parameter for estimation of liver damage (hepatocyte necrosis). This enzyme and additional laboratory parameters should be initial steps for estimation of liver disease in HIV patients with hepatomegaly. Many authors agree that elevated serum activity of the two commonly used liver enzymes (ALT and AST) that are involved in breakdown of amino acids reflects liver cell injury [7-9].

Histological assessment of liver tissue in patients with HIV infection showed three particular changes: non-specific alterations, granulomatosis, and previous liver diseases (i.e. opportunistic tumors, toxic lesions, etc.). The non-specific alterations depended on the grade of the impairment of host cellular immunity (CD4 T lymphocyte count). The relationship of non-specific histological alterations such as hyperplasia and hypertrophy of Kupffer cells and polyclonal stimulation of B lymphocytes with production of antibodies is not fully understood. Some authors reported these changes as probably caused by repeated infections or by potential consequence of the virus persistence in Kupffer cells [10]. The majority of authors agree that dilatation of sinusoids in liver tissue precedes Kaposi's sarcoma [1, 2]. An additional finding in HIV infection is the low level of hemoglobin caused by phagocytosis of erythrocytes that has been seen only in AIDS patients.

Our investigation revealed presence of liver granulomas in HIV-infected patients. Mycobacterium tuberculosis, that can induce fatal outcome in HIV patients if not treated properly, was found in 18% of tissue samples. Characteristic features of CMV infected cells formatting granulomas were seen in 7% of liver tissues. Although we also noticed granulomas

consisting of micro-crystals with talc in intravenous drug abusers, there was a significant frequency (15.6%) of liver granulomatosis with unknown etiology.

Serological analysis showed that half of the HIV patients with liver enlargement had HCV infection. A high frequency of infection with this virus was previously reported [11-13]. The histological changes in our patients confirmed infection with HBV and HCV in most patients causing chronic liver diseases. The histological features of chronic hepatitis B depended on the degree of cellular immunity and response. Patients with AIDS and extremely low cellular immune defense expressed milder histological changes of chronic hepatitis, while in the patients with more competent immunity, moderate to severe presence of hepatocyte necrosis and inflammation were seen. However, different histological types of chronic viral B and C hepatitis were evidenced, from minimal and mild grade (activity) and no or mild stage (fibrosis) to established liver cirrhosis.

We observed that the most frequent bacterial infection of the liver was MT, detected in 43.4% of liver granulomas without caseous necrosis. This finding is in accordance with other studies [14-17]. Furthermore, it was reported that a focal form of tuberculosis of the liver was rarely diagnosed [18]. In this study, we discovered only one case with a focal form of tuberculosis. Although accepted as a common pathogen in AIDS producing disease of the central nervous system, *Cryptococcus neoformans* was detected in the liver only in 2.3% of the patients.

Finally, the most frequent opportunistic infections in AIDS patients with hepatomegaly were HCV and MT, while HCV and HBV were mostly detected in patients without AIDS. Simultaneous infection with hepatitis B and C viruses was exclusively found in AIDS patients. Recent studies reported an increasing rate of liver disease in AIDS patients, caused by hepatotropic viruses and related to death [13, 19-21]. In our previous report, one of the serum HBV markers was initially detected in 65% HIV-infected patients [22].

In spite of extensive clinical and laboratory investigations, opportunistic infection is not recognized in 5% of HIV-infected patients with hepatomegaly. When the cause of liver disease is unknown, the term "cryptogenic liver disease" - of unknown origin - is used. This condition has never been fully described up to date, meaning that its cause, clinical importance, and prognosis are unknown.

**In conclusion**, liver enlargement is an important clinical feature in patients with HIV infection. Hepatotropic viruses (HCV and HBV) are the main cause of liver enlargement in these patients in association with other opportunistic infections, mainly *Mycobacterium tuberculosis*.

### Conflicts of interest

Nothing to declare.

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