Original Paper

Microscopic findings in liver steatosis in HIV-infected children and dead with AIDS

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ABSTRACT The present histological study followed the presence of liver steatosis in HIV-infected children subsequently dead with AIDS. At autopsy, liver specimens have been sampled from 22 subjects in B and respectively C clinical and immunological stages. The liver specimens have been processed for paraffin embedding, then cut with a microtom into 3-5 micrometer-thick sections, which were stained with haematoxylin-eosin and thricrome Goldner-Szekelly. The presence of liver steatosis was identified in 16 cases (72,8%), in the other 6 cases no steatotic changes being identified. In these 16 cases with presence of liver steatosis, steatosis was graded as follows: mild steatosis in 6 cases (37,5%), moderate steatosis in 4 cases (25%) and severe and massive steatosis in 6 cases (37,5%).The microscopically aspect of liver steatosis, was the most frequently described, as macrovesicular in 8 cases, in 4 cases there were microvesicular changes, and the other 4 cases presented with a mixed aspect, macrovesicular and microvesicular.

KEYWORDS *liver steatosis, HIV, AIDS, lipodystrophy syndrome*

Introduction

Hepatic steatosis is one of the histological changes commonly seen in HIV positive patients, defined as an accumulation of fat in hepatocytes. There are two types of steatosis determined by the way of lipid accumulation inside the hepatocytes: macrovesicular steatosis by a single lipid vacuole wich pushes the cytoplasm and nucleus of the hepatocytes to the peripheral side and microvesicular steatosis characterized by the presence of several lipid vacuoles in the cytoplasm of hepatocytes [1,2]). Steatosis may be caused by different pathogenic mechanisms, especially abnormalities of lipid metabolism, denutrition, toxic injuries and viral infections with hepatitis viruses B or C.But in its pathogenesis may be incriminated HIV infection itself or antiretroviral therapy administrated,especially the ones with nucleoside reverse transcriptase inhibitors (NRTIs) and as well as protease inhibitors(PI).

Antiretroviral drugs allow suppression of viral replication, reducing the incidence of opportunistic infections and prolonged survival of HIV infected patients. But these drugs can be, on the other hand, responsible for lipodystrophy syndrome consisting of metabolic complications and particularly in lipid metabolism and abnormal fat redistribution. The result is loss of subcutaneous fat (lipoatrophy) or regional or generalized, with accumulation of fat in the viscera and especially in the liver and can cause mild gastrointestinal symptoms. The prevalence of lipodystrophy syndrome has been estimated between 30-50% in some studies and a prospective after a period of 18 months after starting antiretroviral therapy indicates a prevalence of 17% [3].

It was found that children,as well as adults, may experience lipodystrophy syndrome, sometimes shortly after initiation of antiretroviral therapy.

Reverstranscriptase nucleoside inhibitors (NRTIs) can cause lipodystrophy by mitochondrial toxicity that is responsible for inhibiting mitochondrial effect. Mitochondrial toxicity is responsible for inhibition of DNA polimerase gamma that will lead to reduced mitochondrial DNA and impaired respiratory chain, which resulted in the following adverse effects: myopathy, hyperlactatemia,and frequently macrovesicular steatosis, and also microvesicular or mixed as well as steatohepatitis with lactic acidosis[3,4].

There is a classification in descending order by their ability to NRTIs inhibit DNA polymerase gamma "in vitro" namely: Stavudine, Didanosine, Zidovudine, Lamivudine and Zalcitabine. More recently, studies of mitochondrial DNA depletion in hepatocytes showed that Zalcitabine, Didanosine and Stavudine have more strength than enzyme inhibition compared with Zidovudine, Lamivudine and Abacavir [5].

Mitochondrial toxicity is NRTI dose-dependent and time management too.

Recent studies show that 40% of HIV-infected patients treated with protease inhibitors (PIs) for a period exceeding more than one year,develops lipodystrophy syndrome [6.7] with the development of hepatic steatosis, but the risk of this syndrome may occur any time during the treatment. Increased risk for hepatotoxicity protease inhibitor therapy is much higher in the pre-existence of chronic hepatitis B or hepatitis C.

Also, the combination of NRTIs with IP obviously increases the risk of hepatic steatosis.

Material and method

Our study aimed presence of liver steatosis on liver fragments collected at autopsy from a total of 22 children aged 5 months to 16 years, deceased of AIDS. Paraffin blocks were sectioned at a thickness of 4 micron sections were stained using hematoxylin-eosin stain and trichrome Goldner-Szekelly stain. The stage of steatosis was assessed by Lucia Rubbia-Brandt [8] and Neau D., Winnock M. et al, 2007 [9]. Steatosis was graded as follows: no steatosis, mild steatosis (1% -10% of hepatocytes), moderate steatosis (11% -30%), severe steatosis (31% -60%) and massive steatosis (more than 60% of hepatocytes).

Clinical study of sheets of deceased children were gathered data on the age at which they deceased, gender, AIDS stage at death; numerical values and percentage of CD4 lymphocytes, the presence or absence of hepatomegaly, the values of hepatic biochemical parameters such as alanine amino-transferase (GPT), aspartate amino-transferase (GOT), direct and total bilirubin, alkaline phosphatase, lactate dehydrogenase (LDH) dysproteinemia liver tests, presence or absence of HBsAg (Ag.HBs), triglycerides and cholesterol values, protein electrophoresis, administration or not of antiretroviral medications during their life.

According the study of pathology records we collected data on pathological examination which was conducted during necropsies.

Results and discussion

The microscopic study of sections stained with hematoxylin - eosin and trichrome Goldner-Szekelly,showed mild steatosis which was found in 6 cases (27.27%) (see Figure 1), moderate steatosis in 4 cases (18.18%), severe and massive steatosis in 6 cases (27.27%) and absence of steatosis was observed in 6 cases (27.27%). Of cases with present steatosis 8 cases (50%) had macrovesicular steatosis, 4 cases (25%) had microvesicular steatosis and 4 cases (25%) had steatosis combined macrovesicular and microvesicular. The distribution of macrovesicular and microvesicular steatosis type depending on the degree of steatosis was as follows:

The 6 cases with mild steatosis:

- 3 cases (50%) had microvesicular steatosis
- 3 cases (50%) had macrovesicular steatosis

The 4 cases with moderate steatosis:

- 2 cases (50%) had macrovesicular steatosis (fig. 2)
- 1 case (25%) had microvesicular steatosis
- 1 case (25%) showed mixed steatosis microvesicular and macrovesicular

The 6 cases with severe and massive steatosis:

- 3 cases (50%) had macrovesicular steatosis (fig.3, fig.4)
- 3 cases (50%) showed mixed steatosis macrovesicular and microvesicular (Figure 5)

Of the 6 cases with mild steatosis, 4 cases were immunodeficient AIDS being in stage C3 and two AIDS cases were in stage B, or B3 at time of death.

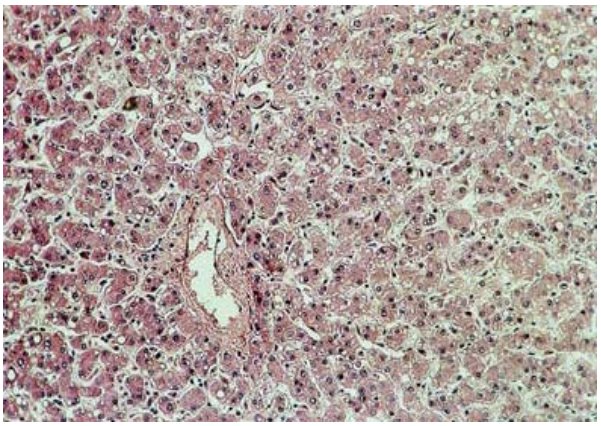

Figure 1. Mild steatosis macrovesicular and microvesicular within a liver lobule. HE stain, ob. x 10

Hepatomegaly was found on physical examination in 5 cases, 3 cases with moderate hepatomegaly, lower edge of the liver was approximately 1.5 to 2 cm below the costal margin, the other 2 cases with a high degree of hepatomegaly lower edge of the liver to 3 cm below the costal margin. In all 6 cases the children were malnourished.

Regarding hepatic transaminase value: 2 cases (33.3%) had values of transaminases, especially the GPT normal and 4 cases (66.7%) had increased GPT values from 1.25 to 2 , 5 times the normal value accompanied by altered hepatic tests. Only one case of those with high transaminases had increased alkaline phosphatase in the other 3 cases it was normal. Total bilirubin was normal in all 6 cazuri. One case (16.66%) was

chronic carrier of HBs antigen. Three cases (50%) received antiretroviral treatment, and of these 3 cases one is that chronic carrier of HBs antigen presenting therefore an increased risk of steatosis. Two cases received NRTIs and non-nucleoside reverse transcriptase inhibitors (NNRTIs), and one case received a combination of NRTIs, NNRTIs and protease inhibitors (PIs).

Of the 4 cases with moderate steatosis all 4 cases were classified as C3 AIDS stage in terms of clinical and immunological classification. In all 4 cases the children were malnourished. Hepatomegaly was present in all 4 cases, 2 cases were moderately lower edge of the liver at 1-2 cm below the costal margin, and in 2 cases hepatomegaly was high, with the lower edge of the liver at 3-4 cm below rebord. Chronic HBs antigen porting was found in 2 cases (50%). Increases of GPT of 1.25-2.5 times the normal value were present in all 4 cases, 2 cases were accompanied also by high alkaline phosphatase, and in one case by total bilirubin increasing. Two cases (50%) received antiretroviral treatment, one of them is actually the one who was HBsAg chronic carrier, which thus presents a high risk for developing liver steatosis. One case received NRTIs, NNRTIs and PIs and the other received NRTIs and NNRTIs. Both cases received Stavudine.

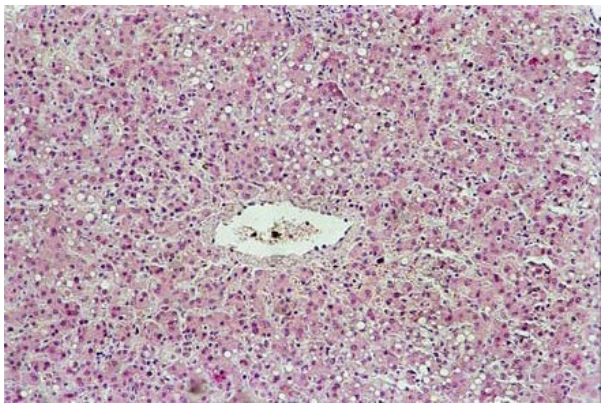

Figure 2. Moderate macrovesicular steatosis on the liver lobule. HE stain, ob. x 10

Of the 6 cases (27.27%) of children with severe and massive steatosis, 3 cases were B2 stage at the time of death in terms of clinical and immunological classification, a case was in B1 stage, a case in progress C1 and C2 stage event. All 6 children were malnourished. Hepatomegaly was present in all 6 cases, 2 cases with mild hepatomegaly approximately 1 cm below the costal margin, the other 4 cases showing a high degree of hepatomegaly lower edge of the liver at 3-4 cm below rebord. Chronic HBs antigen porting was found in 3 cases (50%). No case did receive antiretroviral treatment. In 3 cases (50%) there were met increased values of GPT, in 2 cases with values ranging from 1.25 to 2.5 times normal and in one known case of toxic hepatitis drug. GPT had 100 times normal values, 3 cases (50%) had normal hepatic transaminases.

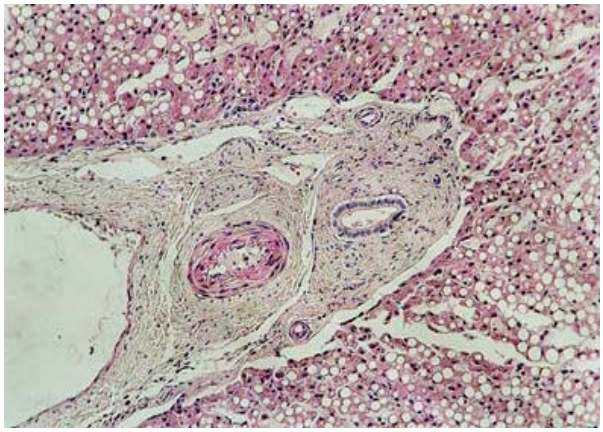

Figure 3. Severe macrovesicular steatosis to periphery of hepatic lobules near by a portobiliar area that has a massive fibrosis. HE stain, ob.x 10

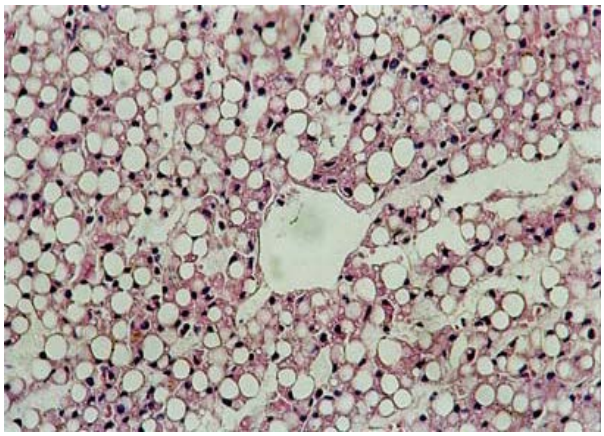

Figure 4. Massive macrovesicular steatosis arround of middle lobules vein. HE stain, ob. x 20

Figure 5 Severe macrovesicular steatosis on the rebord of the hepatic lobule. Trichrome Goldner-Szekely stain, ob.x 10

Hepatomegaly was found on physical examination in 5 cases, 3 cases with moderate hepatomegaly, lower edge of the liver was approximately 1.5 to 2 cm below the costal margin, the other 2 cases with a high degree of hepatomegaly lower edge of the liver to 3 cm below the costal margin. In all 6 cases the children were malnourished.

Of the 6 cases without steatosis, a case was clinical and immunological B3 stage at the time of death, a case in C stage, and the other 4 cases were in an advanced C3 AIDS stage.

Hepatomegaly was present in all 6 cases,in one case being very low, the costal margin in the other 5 cases were high-grade hepatomegaly with lower edge of the liver at 3-4 cm and 5 cm below rebord. HBsAg was present in 3 cases (50%) who did not receive antiretroviral treatment.Two cases (33.33%) received antiretroviral treatment. Transaminases have been modified in 3 cases (50%), SGPT values were increased to values approximately 2-fold normal, and in one of these 3 cases showed very high GOT 15 times normal (3oo IU / L); 2 cases (33.3%) had normal levels, and in one case they were not made. Malnutrition was present in 3 cases (50%).

Pre-HAART era liver steatosis was observed in 30-50% of HIV-infected patients [10]. Percentages between 3-57% have been reported in other studies. Schneiderman DJ. and his collaborators [11] and Lebovics E. et al. [12], appreciated steatosis as being present in a proportion of 42% and 56% respectively in those cases was the appearance of macrovesicular steatosis and BJ Glasgow. et al. [13] reported it to be present in a proportion of 57%.

In most cases, the cause of steatosis was poor nutrition.

In our study, steatosis was observed on sections stained with hematoxylin-eosin and trichrome Goldner-Szeckelly in 72.7% of cases, the increased percentage to these studies in the speciality literature.

An recent study conducted on a group of 27 children with HIV infection, however, shows a higher prevalence of nonspecific hepatic steatosis at these children, up to 85% [14], without a clear relationship to antiretroviral treatment. An explanation of these cases could be advanced HIV infection which is associated with increased release of alpha-TNF that can weaken mitochondrial respiration and block the respiratory chain, increasing the formation of reactive oxygen species (ROS) and lipid peroxidation.

On physical examination, hepatomegaly, moderate in most cases, was observed in 21 out of the 22 cases studied (95.4%), only one case with mild steatosis not producing hepatomegaly. This is in accordance with results of other studies that assessed hepatomegaly as being present in a proportion of about 90% in HIV positive patients with hepatic steatosis. [15]. Hepatomegaly was often accompanied by changes in the consistency of the liver, which appeared of increased consistency with sharp edge and sensitive to touching.

According to current studies [16,17,18], hepatic steatosis is clinically asymptomatic or associated with minimal gastrointestinal symptoms,wich was noticed by us at our studies cases, one case with massive steatosis but showing signs of hepatic failure with very high GPT of 100 times than normal due to toxic hepatitis drug.

Diagnosis of hepatic steatosis is often put on the account paraclinical liver enzymes increasement. An increase of their values and especially GPT values over 2-3 times than normal is often encountered, although some patients may have normal values [19]. Increased by 2-3 times over the normal limit of transaminases and in particular of GPT were observed in 11 cases (68.7%) of the 16 cases with steatosis present, the other 5 cases with normal values. We found, however, that GPT values were not correlated with the stage of steatosis, only 3 out of the 6 cases with severe and massive steatosis had increased values, the other 4 cases being normal. If moderate steatosis all 4 cases had increased levels of GPT, and for mild steatosis 4 out of the 6 cases (66.7%) had increased GPT values, and the others normal values.

On the other hand, the 6 cases without hepatic steatosis, 50% (3 cases) had high GPT values 2 times than normal. This may be due to HIV co-infection in these cases with hepatitis viruses B and / or C, to other opportunistic infections overlay or to other potentially hepatotoxic drugs taken for other diseases of the patients. We thus conclude that the values of transaminase can not be considered a typical screening test to assess the presence of steatosis.

Out of the 16 cases where steatosis was present, in 6 cases (one case of mild steatosis, 2 cases with moderate steatosis and 3 cases with severe steatosis) was found the presence of HBsAg which is an indicative of chronic hepatitis with hepatitis B virus . This is in accordance with data from the speciality literature which considers the presence of hepatic steatosis in chronic hepatitis B virus at a rate between 27-51% [20].

Trying to find the causes of hepatic steatosis in the studied cases according to the stages of steatosis, we found that:

On the 6 cases (27.27%) with mild steatosis, it was due to:

• in a case-patient was chronic carrier of HBsAntigen and received antiretroviral treatment
• 3 cases - they received antiretroviral treatment

• 2 cases were due to HIV infection itself, it is not mentioned other possible causes, but it could be determined by other medicines for other diseases that could cause the appearance of steatosis.

In the 4 cases (18.18%) with moderate steatosis, steatosis was due to:

• In 2 cases, the presence of chronic hepatitis with HBs antigen associated with antiretroviral therapy
• 2 cases-infection with HIV or other drugs medicine used for other diseases

On the 6 cases (27.27%) with severe steatosis or massive steatosis may be due to:

• In 3 cases, chronic hepatitis with HBs antigen present

• In one case of toxic drugs induced hepatitis - other medicines drugs used for other diseases (tuberculostatics, antifungal, Biseptol), knowing that in this case it was not given any antiretroviral treatment

• 2 cases, HIV infection or other potentially hepatotoxic drugs administered.

Regarding antiretroviral treatment in the 16 cases with steatosis present, it was administered only in 5 cases (31.25%),out of which 4 cases had been given Stavudine, and among those with severe steatosis no case was treated with antiretrovirals. Also, the 6 cases without steatosis, 2 cases were given antiretroviral medication, including Stavudine.

Conclusions

On our processed study the following conclusions can be drawn. Hepatic steatosis was observed in 72.7% of studied cases. Hepatomegaly is frequently observed at children with hepatic steatosis. It was found that hepatic enzymes may be used as a screening test to assess the presence of common hepatic steatosis, there are cases of steatosis that their values were within normal limits. In group studied, antiretroviral therapy was administered only 31.25% of cases with steatosis present. Of the 6 cases without steatosis, 2 cases received antiretroviral treatment and in these cases it was taken into account that mitochondrial toxicity and hepatic steatosis induced by antiretroviral therapy depend on the administrated dose and of duration of administration of the therapy.

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