Characteristics of Hepatitis B Co-infection and Disease Evolution in HIV-Positive Paediatric Patients in Romania

Manuela Arbune¹, Costinela Georgescu²

¹Department of Morphological Sciences, Dunarea de Jos University Faculty of Medicine and Pharmacy, Galati, Romania
²Department of Pharmaceutical Sciences, Dunarea de Jos University Faculty of Medicine and Pharmacy, Galati, Romania

ABSTRACT

Background: Infection with hepatitis B virus (HBV) contributes to morbidity and mortality in people living with human immunodeficiency virus (HIV).

Aims: The aim of the present study is to assess the influence of HBV co-infection in clinical characteristics and disease evolution among nosocomial HIV infected youth in Romania.

Study Design: Retrospective study.

Methods: We assessed HBsAg in 179 young people with nosocomial paediatric HIV infection. Demographic data, ALAT level, CD4-count, HIV-RNA, antiretroviral therapy and clinical behaviour were all statistically compared in patients who were HIV mono-infected and HBV-co-infected.

Results: The characteristics of patients are as follows: sex ratio M/F: 55.3%, AIDS category 88%, median nadir CD4-count 126/mm³. The prevalence of persistent HBsAg was 44.6%. The mortality rate was 11.1%, but no correlation with HBsAg was found. An average of three antiretroviral combinations is experienced by 97.7% of patients, including Lamivudine for over 5 years in 76% of cases and Tenofovir/Emtricitabine in 16.75% of patients. Patients under antiretroviral therapy achieved 53.07% sustained undetectable HIV-RNA and 40.78% restored immunity CD4-count >500/mm³. ALAT enzyme was found to be high in 54.75% of patients.

Conclusion: During our research, we noticed that HBsAg was elevated in young people with HIV in Romania. Mortality rate was not statistically correlated to HBsAg. High ALAT levels are related with HBV, HDV co-infections, virological failure to antiretroviral treatment and the risk of death.

Key Words: HIV, Hepatitis B, antiviral agents, Romania

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Introduction

Human Immunodeficiency Virus (HIV) and Hepatitis B Virus (HBV) share similar transmission routes. The risk of chronic hepatitis B is higher in co-infected HIV patients, while acute HBV mono-infection is self-limited in 90% of adults (1). Although efficient HBV vaccination is available, over two thirds of people with HIV have a serological marker of HBV infection (2). Across the European region, the prevalence of HBV varies, from low rates in Western countries (<2/100,000) to high rates in Eastern countries (>8/100,000) (3, 4).

A high incidence of hepatitis B, especially in children, was reported in Romania twenty years ago. Due to continuation of a routine immunisation program for newborns - acting since 1995 - the incidence of hepatitis B decreased significantly between 1989 and 2004, from 43/100,000 to 8.5/100,000 (5). The peculiarity of HIV epidemics in Romania is represented by the predominance of a unique paediatric group of patients born between 1988 and 1990 that presented nosocomial infection during the first years of life. These children infected with HIV have grown into present-time adults with an active sex life fact that implies risks for spreading HIV and other sexually-transmitted diseases. The fact that many of these children survived for a long period of time could be related to antiretroviral therapy (ART) and to a prevalent HIV-F subtype (6). According to an epidemiological study of teenagers with HIV in Romania, 78% of HIV patients of this paediatric group had HBV markers of past or present infection and 43.4% had HBV replication markers (7).

The reciprocal impact of HIV and HBV requires a special approach towards the HIV-HBV co-infected population. Hepatitis B tends to be more severe in HIV-positive patients and increases the risk of mortality by liver diseases, as well as leading to the rapid progression of immunodeficiency (8). High HBV replication with consequent severe fibrosis is frequently related with HIV, while low or middle necroinflammation can be currently observed. HBV reactivation is more likely to occur in HIV patients even after HBs antibody response (1, 8). Hepatitis delta virus (HDV) represents a risk factor for the severity of chronic hepatitis B, but was not associated with progression to the stage of acquired immunodeficiency syndrome (AIDS) (9).

Most clinical trials have not clearly proven the role of HBV in HIV progression. However, there have been reports of higher risks of hepato-toxicity under ART and hepatic flares when ART is interrupted in HIV-HBV co-infected patients (10).

A group study on the meta-analysis of HBV infection regarding the mortality in HIV infected persons reported a
The follow-up of this study, we developed a CD4 count tech-
sAg over six months, detected in two or more samples. During
Galati. Persistent HBV infection was defined by positive HB-
assay in the Laboratory of Infectious Diseases Hospital from
HBsAg were tested by means of automatic immune-enzymatic
(NV) were considered high ALAT values. Liver enzymes and
2011 or the date of death. Values over 1.5 normal references
HIV-ARN viral-load and ART drug combinations had been re-
therapy (ART), nadir of CD4-count, clinical and immunologi-
ical HIV/AIDS stage by CDC-1993 criteria, were all collected
in our database (January 2005) (16). We compared the char-
acteristics of patients with positive and negative HBsAg. Ala-
ine Amino-transferase (ALAT) level, HBsAg, CD4-count, and
HIV- ARN viral-load and ART drug combinations had been re-
corded every 6 months. The study endpoint was in December
2011 or the date of death. Values over 1.5 normal references
(NV) were considered high ALAT values. Liver enzymes and
HBsAg were tested by means of automatic immune-enzymatic
assay in the Laboratory of Infectious Diseases Hospital from
Galati. Persistent HBV infection was defined by positive HB-
sAg over six months, detected in two or more samples. During
the follow-up of this study, we developed a CD4 count tech-
nique by means of flow cytometer (FACS Calibur) and quan-
titative analysis of HIV-RNA viral load in blood by polymerase
chain reaction (COBAS TaqMan HIV-1), both of which were
performed in The Clinical Laboratories of National Institute
for Infectious Diseases “Prof. Dr. Matei Bals” from Bucharest.
The HIV-RNA undetectable level was considered below 400
copies/mL.

Updated ART was decided according to European and
Romanian HIV therapeutic guidelines (12,17). The ART levels
that were studied were counted at the end of the study as
the number of ART combinations and cumulative individual
therapy period (month basis). We assessed the influence of
common HIV and HBV antiviral drugs on HBsAg seroconver-
sion, elevated ALAT levels and mortality rate, as the endpoint.
The study was approved by The Ethical Committee of The
Infectious Diseases Clinic of “Dunarea de Jos” University from
Galati.

Data was analysed by the XLSTAT statistical software. Mean or median values were calculated for continuous vari-
ables. The relationships between categorical variables were
described with the cross-tabulation technique. Chi square test
was used to examine the association between two categori-
variables between groups. The results were processed as
odds ratios (OR) and 95% confidence intervals (CI), with statis-
tical significance levels of p<0.05.

**Results**

Persistent positive HBsAg was found in 44.6% (80/179) HIV
patients from the paediatric cohort. Positive and negative HB-
sAg groups have similar distribution by sex and educational
level. Most HBsAg positive patients are living in urban areas.
Eighty-eight per cent of patients had documented clinical and/
or immunological AIDS criteria, but progression to AIDS was
not significantly influenced by the persistent HBsAg (Table 1).

Global mortality rate was 11.1% (20/179), but the rate of
death was not related to HBV co-infection (p=0.654). The se-
roconversion rate of HBsAg was found to be 17.5% (14/80) in
HIV-HBV co-infected patients. Although HBs seroconversion
was not noticed in any deceased patients, the death rate was
not statistically influenced (p=0.169).

Evaluation of HDV markers was attained by 67/80 patients
with persistent HBsAg; 16/67 were found to be positive. Co-
replication of both HBV and HDV might enhance liver injury
and significantly elevate ALAT, concordant with our findings
(Table 2).

An average of three (1, 9) ART combinations were expe-
rienced by 179 of the patients until the end of 2011, includ-
ing nucleoside/nucleotide class of common drugs for HIV
and HBV, such as Lamivudine (LAM), Tenofovir (TDF) and
Emtricitabine (EMC). Lamivudine was the most extensive expe-
rrienced drug from the beginning of ART history while TDF
and EMC became available for HIV treatment in Romania after
2010. In our study, 83% of patients were treated with LAM
and 76% had over 5 years of LAM experience. Tenofovir and
EMC were used together for most of the ART combinations
and were supplied to 16.75% of patients. Replacing LAM with
TDF/EMC was chosen for both HIV and HBV, according to the
European AIDS Clinical Society algorithm of treatment in HIV/
HBV co-infection (12).

Both LAM and TDF/EMC have been administered in com-
binations that had at least two nucleoside reverse transcript-
tase inhibitors (NRTI) and a boosted protease inhibitor (PI/r) or
non-nucleoside reverse transcriptase inhibitors (NNRTI). Some
other drug classes were rarely combined, and this only hap-
pened in patients requiring salvage therapy.

Antiretroviral therapy evaluation at the end of this study
emphasises that 53.07% of patients had sustained undetect-
able HIV-RNA and 40.78% had restored immunity with CD4
>500/mm^3. Effectiveness of ART is independent of HB-
sAg. Elevated ALAT was recorded in 54.75% of patients, but
only 28% scored three times over the normal value (NV). None
of the 19 available NRTI, NNRTI and PI agents used for ART combinations were related to significant liver toxicity. It was also observed that ALAT levels increased more frequently in patients that stopped using LAM than in those who had ongoing LAM therapy (p=0.001; OR=4.58). Increased ALAT can be correlated with HBsAg, HDV co-infection, virological HIV failure on ART and HIV-related mortality. However, high ALAT values are more commonly in patients with restored immunity of CD4-count over 500/mm³, which is probably explained by the pathology of immune reconstruction following ART (18).

Increased serum ALAT levels are not statistically correlated with common risk factors such as alcohol, smoking, recreational drugs or concomitant anti-tuberculosis drugs for hepatotoxicity (Table 2).

Discussion

The high frequency of HIV/HBV co-infection in Galati is typical for Romanian paediatric HIV epidemics. Considering that HIV infection was transmitted in early childhood, diagnosis of the disease varied depending on the age of the patient, on other associated diseases and on the individual context.
Taking into account the associated HIV-related depressed immunity and frequent immaturity of the immune system during early childhood, a high rate of HBV persistence is expected in spite of long periods without symptoms. Compared with EuroSIDA Cohort data (16,505 patients), the proportion of HIV/HBV co-infection in our study is over six times higher: 44.6% vs. 7.1% (2). Testing methods for HBsAg and hepatitis C virus (HCV) antibodies are routine practices for a basic evaluation in newly diagnosed HIV patients (12, 17). Other investigations recommended by current guidelines for the management of HIV/HBV co-infection, such as HBV-DNA, non-invasive histological tests or other HBV and HDV serological markers, are not made available by the Romanian health insurance system in our clinic, thus our patients can rarely have them done. Assessment of HBV co-infection among HIV patients by HBsAg is not accurate enough for severe immunosuppressed conditions (1).

Co-replication of both HBV and HDV might enhance liver injury and significantly elevate ALAT, concordant with our findings. The frequency of HIV/HBV/HVD co-infection among young people in Romania is 1.64 times higher than EuroSIDA Cohort data: 23.8% vs. 14.5% (2). The assessment of HDV influence on mortality rate is not feasible because our deceased patients were not previously tested for this virus. Mortality is independent of HBV in our study, but the EuroSIDA study has reported a six times higher mortality rate related to HBV (2). This discrepancy can be explained by age differences between Romanian patients and those in the EuroSIDA Cohort report and also by the variable virulence of HIV and HBV subtypes present in European countries.

Long-term HIV infection in young people in Romania in the paediatric cohort shows the HIV/AIDS history from the last 23 years. Survival is the most important achievement of ART, although history recalls many errors that were made at the beginning of HIV therapy. The primary aim of ART combination, including LAM, was to suppress HIV replication and not necessarily HBV. The apparent influence of LAM on HBV was observed in 17.5% of HIV/HBV co-infected patients with HBs seroconversion. However, HIV resistance mutations can have a quite fast response, especially in non-adherent patients. Moreover, LAM benefits in the prevention and limitation of liver injuries depend on time response (15). Using LAM in patients with previous multiple therapies with resistant strains is a treatment strategy to maintain both HIV and HBV “viral fitness” when there are no other treatment options. Although current international guidelines recommend the use of TDF and EMC in the first line of ART, they were only recently made available for our patients and are usually used in salvage therapy. Lamivudine was changed with TDF/EMC in thirty of our patients with ART failure and apparent HBV resistance (17). The testing procedures for HBV resistance in LAM therapy was not available for our patients. The assumption of Lamivudine resistance was based on long-term exposure to LAM and high ALAT levels. Normalisation of serum ALAT levels, without significant kidney toxicity, succeeded after six months of TDF/EMC therapy. Future screening of TDF/EMC patients as part of ART should be carefully taken into account for both HBV and HIV response. Discontinuity of TDF/EMC and HBV drug-resistance are related to hepatitis flare ups. The present peculiar aspects of HBV co-infection among Romanian youths call for a review of the therapeutic strategy. Up to date investigations for viral hepatitis diagnostic, new antiviral agents and liver transplants are future expectations for the efficient management of HIV/HBV co-infection.

In conclusion, the prevalence of persistent HBV is 44.6% on youth in HIV paediatric cohort in Galati. Mortality rate in HIV patients is not influenced by HBV co-infection. Long-term treatment for HIV with Lamivudine overlaps with HBs seroconversion in 17.5% of HIV/HBV co-infected patients. Immunological and HIV-virological failure under ART, the interruption of Lamivudine, HBV and HVD co-infections are predictable factors for increased ALAT values. The accurate assessment of HBV infection is necessary in order to improve the management of HIV/HBV co-infected young people in Romania.

**Ethics Committee Approval:** Ethics committee approval was received from “Dunarea de Jos” University from Galati.

**Informed Consent:** Written informed consent was waived because of the retrospective nature of the analysis but all patients previously permitted to the use of their medical records for the purpose of any form of clinical study.

**Peer-review:** Externally peer-reviewed.


**Conflict of Interest:** No conflict of interest was declared by the authors.

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