Association of hepatitis B infection in patients with HIV Encephalopathy

Received for publication, August 20, 2012
Accepted, December 15, 2012

PAUL MARINESCU¹, LOREDANA SABINA CORNELIA MANOLESCU²*
¹MD, PhD, assistant professor of virology, Public Health Direction, Giurgiu, Romania,
²MD, HIV/AIDS coordinator for Giurgiu County, Carol Davila University of Medicine and Pharmacy, Stefan S. Nicolau Institute of Virology, Bucharest, Romania.
*Corresponding author: Loredana Sabina Cornelia Manolescu, MD PhD, Carol Davila University of Medicine and Pharmacy, Stefan S. Nicolau Institute of Virology, 285 Mihai Bravu, Bucharest, 030304, Romania, tel: 0040723699253; fax:0040213242590, manolesculoredana@yahoo.com

Abstract

Objectives: Human immunodeficiency virus (HIV) can affect the central nervous system and determine HIV encephalopathy (HE). Evidence of hepatitis B virus (HBV) was found in cerebrospinal fluid in HIV co-infected patients. Here we assessed the degree of association between HBV infection and prognosis of HE in a large cohort of 462 HIV infected patients over a ten years period and the role of nadir CD4 cell count.

Materials and methods: HIV encephalopathy, HBV infections markers, HIV RNA and CD4 cell were measured and retrospectively analyzed.

Results: The prevalence of HE was 22.7%. More than half, 50.4% of the patients with HE presented HBV infection. Among the fifty three patients that presented at the same time HE and HBV infection and prognosis of HE in a large cohort of 462 HIV infected patients over a ten years period and the role of nadir CD4 cell count. The results are as follows:

Materials and methods: HIV encephalopathy, HBV infections markers, HIV RNA and CD4 cell were measured and retrospectively analyzed.

Results: The prevalence of HE was 22.7%. More than half, 50.4% of the patients with HE presented HBV infection. Among the fifty three patients that presented at the same time HE and HBV infection, more than half, 66.03%, were first infected with HBV and then developed HE. It is possible that HBV infection is a risk factor for developing of HE. Further studies are needed to prove the HBV neurotropic potential.

Conclusions: The prognosis of HE was not significantly different in HBV presence or under antiretroviral treatment. Absolute CD4 nadir count and class C3 are proved to be strong predictors of HE in HIV infected patients even after several changes in antiretroviral therapy schemes.

Key words: Class C3, HBV infection , HIV, HIV encephalopathy (HE), nadir CD4

1. Introduction

Human immunodeficiency virus (HIV) can affect the central nervous system (CNS) in the early stages of disease and determine HIV encephalopathy (HE), a complex syndrome with cognitive, motor, and behavioral features (K. GOODKIN & al. [1], W.G. BRADLEY & al. [2]). Sometimes in untreated patients, HE is a part of the acute HIV syndrome. In children treated with highly active antiretroviral treatment (HAART), HE may be infrequent and largely reversible (K. GOODKIN & al. [1]. While the main risk factors associated with HE are well known and include low weight, anemia, low CD4+ count, high plasma HIV-RNA load, hepatitis C virus (HCV) infection and female gender (Y. STERN & al. [3], J.C. MCARTHUR & al. [4], X. LIU & al. [5]), no association with hepatitis B virus (HBV) infection was made. Hepatitis B virus infection is an important cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma worldwide (W.H. SHENG & al. [6]). HBV was found in different extra hepatic sites and the presence of HBV-DNA in CSF of HIV co-infected patients was
previously reported (S. RUTA & al. [7]). Still there is little evidence regarding the
neurotropic character of this virus.
There are 10,903 people living with HIV/AIDS in Romania, according to the data provided by
Compartment for Monitoring and Evaluation of HIV/AIDS Data, National Institute of
Infectious Diseases Prof. Dr. Matei Bals, as of 31st of December 2011. Currently, Romania
has a large number of survivors, integrated in the 19-24 age group, who belong to the 1987-
1990 cohort (>6000). Almost 10% of them are currently living in the same region, the Giurgiul
County (CNLAS [8]).
Here, we assessed the degree of association between hepatitis B virus infection and prognosis
of HIV encephalopathy in a large cohort of HIV-infected patients with common
epidemiological particularities which have experienced multiple therapeutic schemes over
time. We undertook a critical review of the available evidence concerning whether the nadir
CD4 cell count predicts neurocognitive impairment in HIV infected patients after several
changes in antiretroviral therapy schemes.

2. Materials and methods

2.1. Ethics.

This research was approved by the ethical review board of the Infectious Disease Hospital
from Giurgiu County.

2.2. Study participants.

Data from a cohort of 462 HIV infected individuals from Giurgiu County, Romania,
was retrospectively analyzed at different points in time. These patients were born in poor
families or abandoned in foster care during the period from 1987 to 1990 and were probably
infected in the same way and probably during the same period of time, parenterally, by
horizontal route. The main inclusion criteria for selecting these patients from Giurgiu
Infectious Diseases Hospital’s database were HIV infection and the year of birth, making our
cohort very homogeneous. The majority of patients were HIV diagnosed from 1989 to
1999 but there were 35 patients that were diagnosed later between 2000 and 2005. The HBV
diagnosis was performed from 1996 to 2006. The patients were clinically evaluated in day
clinic from Giurgiu Infectious Disease Hospital.
First CD4 and HIV RNA evaluations were performed in 1995 for those already diagnosed and
which started at that time antiretroviral (ARV) therapy. Patients diagnosed after 1995 have
been evaluated at the time of HIV diagnose and if eligible they started ARV therapy. As ARV
drugs developed worldwide they were administered to Romanian patients so their ARV
therapy changed along with the improvement of those drugs and according with the need of
the patients. From the initial group 33.76% patients died until 2006.

2.3. Quantitative CD4 and HIV RNA Parameters.

HIV RNA and absolute CD4 cell count were measured in all the participants enrolled
in the study. Absolute CD4 cell count has been performed at least twice a year and HIV RNA
once a year up to now starting with 1995.
From 1995 and until 2005 absolute CD4 cell count was determined from fresh whole blood
samples using the TRITEST three-color reagent CD4/CD8/CD3 with TRU-COUNT tubes
(BECTON & DICKINSON, USA). Plasma HIV RNA levels were measured from fresh blood
specimens using the ROCHE AMPLICOR, version 1.5 (dynamic range, 400–750 000
copies/mL), manufactured by ROCHE DIAGNOSTIC CORPORATION, USA.
Starting with 2005 absolute CD4 cell count was determined from fresh whole blood samples on a 4-color flow cytometer. Plasma HIV RNA levels were measured from fresh blood specimens using the COBAS AMPLIPREP/COBAS TAQMAN HIV-1 TEST (dynamic range, 20–10,000,000 copies/mL), manufactured by ROCHE DIAGNOSTIC CORPORATION, USA. The tests were conducted in Professor Dr. Victor Babes Infectious Diseases Hospital from Bucharest. Because the dynamic ranges of these assays differed, for combined analyses, we assigned all HIV RNA measurements > 500,000 copies/mL as a value of 500,001 copies/mL, and we assigned measurements <400 copies/mL as a value of 399 copies/mL. Log-transformed HIV RNA levels were used for all analyses.

2.4. Criteria for HIV encephalopathy

HE was defined as the presence, for at least 2 months, of at least one of the following progressive findings in a pediatric patient with no concurrent illness, other than HIV infection, that could explain the findings:

- Failure to attain, or loss of, developmental milestones or loss of intellectual ability verified by standard developmental or neuropsychological tests (Binet-Simon, Portage, Raven, Kids Scid and International HIV Dementia Scale)
- Acquired microcephaly as demonstrated by head circumference measurement or brain atrophy on serial computed tomography (CT) or magnetic resonance imaging (MRI) in children younger than 2 years
- Acquired symmetrical motor deficits manifested by 2 or more of the following: paresis, pathological reflexes, ataxia or gait disturbance (NOMENCLATURE [9]).

In our studied cohort HE was not diagnosed in adult patients.

2.5. HBV Infection Markers

The presence of hepatitis B surface antigen (HBsAg) and total antibody against hepatitis B core antigen (anti HBc) were tested by immunoenzymatic assays (MUREX BIOTECH LIMITED; KENT, ENGLAND) in all patients. The tests were conducted in Professor Dr. Victor Babes Infectious Diseases Hospital from Bucharest.

2.6. Statistical Analyses

Differences in HIV RNA levels and absolute CD4 cell count was evaluated by Fisher's exact test analysis of contingency tables and chi-square test, using GraphPad Prism 5.0. Two-tailed $P$ values were reported. $P < .05$ was considered statistically significant. Mean values were compared using unpaired $t$ test. For scientific significance confidence intervals (CI) were established. $T$ test was performed for categorical variables and $F$ test for continuous variables.

3. Results

3.1. Cohort Characteristics

There were a total of 462 analyzed HIV infected patients. Out of them 156 deceased, median age at death 10 years (limits 7-16). These patients were not treated with any ARV regimen. 197 patients, median age at last investigation 17 years (limits 15-18), were followed up only until 2006 due to several reasons such as: change of residence, lack of adherence or enrollment in other AIDS centers. 109 patients, median age at last investigation 23 years (limits 21-24), were followed up until the end of 2011 (Table 1). All laboratory investigations presented in Table 1 are from their last visit in Giurgiu Day Clinic.

The initial cohort was balanced with regard to sex but differed substantially with
regard to level of immune status and age. In the deceased group the majority, 62.87% were male participants.

Many of the ARV treated patients have begun therapy since 1995 and experimented between one and six antiretroviral regimens until the end of 2011. The therapeutic regimens prescribed over time were: 2NRTIs (for at least 24 months), 3NRTIs (6 months), 2NRTIs+1NNRTI (17 months), 1NRTI+1NNRTI+1PI (22 months), 2NRTIs+2PIs (48 months). All patients received regimens containing NRTIs (2 or 3) and PI (1 or 2), during the last 12 months and some patients received NNRTI along with NRTI and PI in their regimen. Administered regimens included drugs that penetrate CNS such as stavudine, abacavir, nevirapine, and zidovudine. About 40% of the studied patients received these drugs: 63.8% of the patients with HE and 33% of the patients without HE, p=0.0006.

Table 1. Cohort characteristics at last investigation

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total of patients 462</th>
<th>Deceased ARV naive 156</th>
<th>Lost from evidence ARV treated 197</th>
<th>ARV treated 109</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females, no (%)</td>
<td>204 (48%)</td>
<td>58 (37.1%)</td>
<td>112 (56.8%)</td>
<td>61 (55.9%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>17 (7-24)</td>
<td>10 (7-16)</td>
<td>17 (15-18)</td>
<td>23 (21-24)</td>
</tr>
<tr>
<td>CD4 count (cells/µL)_a</td>
<td>242 (0-1652)</td>
<td>73 (0-1040)</td>
<td>379 (5-1652)</td>
<td>495.5 (3-1250)</td>
</tr>
<tr>
<td>HIV RNA Level Log10 (copies/ml)</td>
<td>3.31 (2.6-5.7)</td>
<td>5.5 (3.5-5.7)</td>
<td>2.9 (2.6-5.7)</td>
<td>2.6 (2.6-5.01)</td>
</tr>
</tbody>
</table>

NOTE. Values are medians (limits) unless otherwise listed.

a – P < .0001 by Fisher's and chi-square test.
b – Log10 (copies/mL): values truncated the narrowest dynamic range of 2.6–5.7.

Because the presence of HBsAg was assessed until the end of 2006 and in order to assure a constant number of studied patients for establishing a potential correlation between HBV presence and HE we decided to retrospectively evaluate all 462 patients only for a period of ten years: 1996-2006.

3.2. **HE and HBsAg association**

The prevalence of HE in our studied cohort over a period of ten years was of 22.7% (105 from 462 patients). Surprisingly more than half, 50.4% (53 from 105) of the patients with HE were HBV infected and presented HBsAg (Table 2). The median age of HE diagnosis was 10 years and the median absolute CD4 count at HE diagnosis was 75 cells/µL. In order to establish a possible correlation between HBsAg presence and HE we first reviewed and excluded the main risk factors associated with HE at the moment of diagnosis which include low weight, anemia, constitutional symptoms, low CD4⁺ count, high plasma HIV-RNA viral load. No patient was infected with HCV. In the studied cohort, female gender, another known risk factor for HE previously mentioned in the introduction, did not influenced the analysis, only 44.1% of the cohort was female gender.
Table 2. Demographic, clinical, treatment and laboratory correlation between patients with and without HE, (median values ±SD, unless otherwise listed)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total (n=462)</th>
<th>Patients with HE (n=105)</th>
<th>Patients without HE (n=357)</th>
<th>P value *</th>
<th>OR (95%CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DEMOGRAPHIC PARAMETERS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at HIV diagn. years</td>
<td>7±3 years</td>
<td>7±3 years</td>
<td>7±3 years</td>
<td>0.5389</td>
<td>t=0.6150/df=412</td>
</tr>
<tr>
<td>Age at Hepatitis B diagn. years</td>
<td>11±3.1 years</td>
<td>11±2.9 years</td>
<td>11±3 years</td>
<td>0.7273</td>
<td>t=0.3492/df=189</td>
</tr>
<tr>
<td>Female no; %</td>
<td>204; 44.1%</td>
<td>46; 3.8%</td>
<td>158; 44.2%</td>
<td>1</td>
<td>1 (0.6-1.4)</td>
</tr>
<tr>
<td><strong>CLINICAL PARAMETERS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBsAg positiv, no; %</td>
<td>189; 40.9%</td>
<td>53; 50.4%</td>
<td>136; 38%</td>
<td>0.1</td>
<td>0.7 (0.5-1.1)</td>
</tr>
<tr>
<td>Class C3, no; %</td>
<td>217; 46.9%</td>
<td>82; 78%</td>
<td>135; 37.8%</td>
<td>0.0001*</td>
<td>0.4 (0.3-0.6)</td>
</tr>
<tr>
<td>ARV treated, no; %</td>
<td>306; 66.2%</td>
<td>70; 66.6%</td>
<td>236; 66.1%</td>
<td>1</td>
<td>0.9 (0.7-1.3)</td>
</tr>
<tr>
<td>Treat No. CNS penetr. %</td>
<td>208; 45%</td>
<td>46; 43.8%</td>
<td>162; 45.3%</td>
<td>0.9</td>
<td>1 (0.6-1.5)</td>
</tr>
<tr>
<td>Patients that deceased, no; %</td>
<td>156; 33.7%</td>
<td>56; 53.3%</td>
<td>100; 28%</td>
<td>0.0015</td>
<td>0.5 (0.3-0.7)</td>
</tr>
<tr>
<td><strong>TREATMENT PARAMETERS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4 at baseline, median value±SD, cells/µL</td>
<td>285±352</td>
<td>222.5±333</td>
<td>314±357</td>
<td>0.0663</td>
<td>t=1.841/df=403</td>
</tr>
<tr>
<td>CD4 after 10 years since baseline, median value±SD, cells/µL</td>
<td>242±316</td>
<td>109±291</td>
<td>235±312</td>
<td>0.0257*</td>
<td>t=2.239/df=397</td>
</tr>
<tr>
<td>Nadir CD4, median value±SD, cells/µL</td>
<td>73±198</td>
<td>50±187</td>
<td>102±198</td>
<td>0.0012*</td>
<td>t=3.266/df=402</td>
</tr>
<tr>
<td>HIV RNA Level Log10 median value±SD, (copies/ml) after 10 years since baseline</td>
<td>4.6±5.7</td>
<td>5.2±5.7</td>
<td>3.0±4.9</td>
<td>0.0001*</td>
<td>t=6.118/df=111</td>
</tr>
</tbody>
</table>

*Fisher's exact test, statistically significant; ® Unpaired t test, statistically significant

The patients with HE were divided in two groups: group 1 = patients with HE after the presence of HBsAg, and group 2 = patients with HE, but without HBsAg. Both groups were balanced regarding sex, number of deceased patients, number of class C3 (clinical and immunological AIDS) patients but the patients in group 1 presented lower CD4 values at HE diagnosis than patients from group 2: 44.5 vs 95 cells/µL, p=0.3, lower nadir absolute cell count CD4: 38 vs.51 cell/µL, p=0.1 and slightly higher HIV viral load: 5.2 vs 5 log10 copies/ml, p=0.2.

4. Discussion

Here we evaluated a large cohort of 462 HIV infected patients at the median age of seven years over a period of ten years and we tried to reveal the neurotropic character of HBV virus and the association with HE. In our cohort the median HE diagnosis age was ten years and the...
median hepatitis B diagnosis age was eleven years. There were only fifty three patients that presented at the same time HE and hepatitis B viral infection and more than half, 66.03%, were first infected with HBV virus; even if in the majority of these cases the HE was diagnosed only some month after HBV was diagnosed. The data resulted from our study point to a possible association between HBV and HE. Also there are studies that state that HBV was detected in CSF of the patients with HE (S. RUTA & al. [7]). We believe it may be possible that HBV viral infection is a risk factor for neurological impairment and developing of HE. However, the number of patients is still too little to count for statistical significance even if the initial cohort of patients was large.

In order to see if the presence of hepatitis B viral infection has a different prognosis in HE patients a Kaplan-Meier analysis of the timeline reflecting progression to death was constructed. The analysis did not demonstrate a significant difference according to HBsAg presence ($P = 0.1565$ by the log-rank test) (Fig. 1).

**Fig.1.** Kaplan-Meier Estimates of Survival with Progression to death according to HBsAg presence. The curves represent the percentages of patients surviving with HE during six years after HE diagnosis.

HE affects cognitive, behavioral, and motor function. Prognosis of HE is variable (F.H. BOUWMAN & al. [10]), and depends on antiretroviral treatment. Most of the antiretroviral drugs used to treat HIV in our studied cohort have poor cerebrospinal fluid-to-plasma drug ratios, indicating poor CNS penetration with the exception of stavudine, abacavir, nevirapine, and zidovudine (N.N. SINGH & al. [11]). In the absence of treatment, HE may rapid progress with a mean survival rate of 3-6 months (N.N. SINGH & al. [11]). In the presence of combined antiretroviral treatment cognitive improvement is observed rapidly after initiation and the survival rate may increase from 5 months to 38.5 months (J.C. MCARTHUR & al. [4]). However, recent findings have demonstrated that in a significant proportion of treated patients (V. TOZZI & al. [12], L.A. CYSIQUE & al [13]) recovery is not achieved (V. TOZZI & al. [12], N. SACKTOR & al. [14], V. VALCOUR & al. [15], S.L. LETENDRE & al [16], and A. NATH & al [17]). Currently there are seven approved therapies (D. BHATTACHARYA & al. [18]) for chronic hepatitis B infection based on nucleotide analogues. Several antiretroviral drugs such as lamivudine (3TC), tenofovir (TDF) and emtricitabine (FTC) are used against both HIV and HBV, also included as first-line anti-HIV agents.

In our cohort, HIV/HBV co-infected patients were treated exclusively for HIV and the only drug that was active on HBV viral infection was 3TC and it was administered in an equal
percent in patients with or without HE and could not impair the association between HBsAg and HE. The percent of ARV treated patients was constant for both groups of patients, with and without HE. We could not find any association for the patients treated with drugs that penetrate CNS, because the patients with HE received these drugs in a higher and statistically significant percent, p=0.0006, than patients without HE. The patients treated with CNS penetrating drugs have started ARV therapy before diagnosis of HE.

At the end of the study 46.9% of all patients were considered as class C3 patients. There was a strong association between class C3 and HE: 78% of the patients with HE vs 37.8% of the patients without HE, p<0.0001, were in C3 class.

It is well known that CD4 nadir is a predictor of HE in the presence of ART (J.A. MUNOZ-MORENO & al. [19]) and our study confirmed this statement, there a significant statistical difference in the median absolute CD4 nadir cell count between patients with and without HE: 50 vs. 102 cell/µL, p=0.001. After ten years of evaluation, the median CD4 cell count dropped below 200cell/µL in the HE group.

5. Conclusions

In our studied cohort of HIV infected patients, HBsAg presence was associated frequently with HE but since the number of HBsAg positive patients was not statistically significant further studies are needed to prove the HBV neurotropic potential. The prognosis of HIV encephalopathy was not significantly different in HBsAg presence or under ARV treatment. Absolute CD4 nadir count and class C3 are proved strong predictors of HE in HIV infected patients even after several changes in antiretroviral therapy schemes.

Conflicts of interest. The authors declare that they have no conflicts of interest.

Authors’ contributions. All authors contributed to study design, data interpretation and critical revision of the manuscript.

Acknowledgements. The authors want to acknowledge the medical staff from Infectious Disease Hospital from Giurgiu County and the staff from Sfanta Maria Foundation for providing the patients’ charts.

This paper is supported by the Sectorial Operational Programme Human Resources Development, financed from the European Social Fund and by the Romanian Government under the contract number POSDRU/89/1.5/S/64109

References

Association of hepatitis B infection in patients with HIV Encephalopathy


