

HIV-HBV coinfection and liver fibrosis in long time monitored patients from the former Romanian pediatric HIV cohort – a small study single center experience

Giorgiana-Nicoleta Lungu¹, Florentina Dumitrescu^{2,3}, Anca Duduveche^{2,3},
Andreea Marcu^{2,3}, Lucian-Ion Giubelan^{2,3}

¹Doctoral School, University of Medicine and Pharmacy of Craiova, Craiova, Romania

²“Victor Babes” Hospital of Infectious Diseases and Pulmonology, Craiova, Romania

³Infectious Diseases Department, University of Medicine and Pharmacy of Craiova, Craiova, Romania

ABSTRACT

The aim of this study was to determine the degree of liver fibrosis with the help of a non-invasive method - transient elastography - in a long term monitored HIV-HBV coinfecting group of patients (from the former Romanian pediatric cohort) and to assess the implication of various factors as potential risk factors for the development of liver fibrosis. We have reviewed a total of 636 patients (Px) that were in the evidence of the HIV Department of the “Victor Babes” Hospital of Infectious Diseases of Craiova, from which we randomly selected a group of 37 patients that are monthly attending the HIV department. Mean age of the patients was 34.03±2 years old and they were monitored for a long period of time, mean duration of monitorization was 26.11±4.31 years. Patients have been split into group A (13 Px) with various degrees of liver fibrosis and group B (24 Px) without liver fibrosis; the mean value of liver stiffness was higher in group A vs group B 11.27±5.72 vs.4.93±0.98 kPa, p <0.0001. Mean value of nadir CD4 was lower in group A. No significant differences were found between the 2 groups related to the number of associated risk factors, duration of treatment with Zidovudine, Didanosine, Zalcitabine, Stavudine, non-nucleoside reverse transcriptase inhibitor or Ritonavir. Longer duration with Lamivudine alone was associated with group A, while dual therapy (Tenofovir based) was characteristic with group B. In conclusion, in a prolonged monitoring of HIV-HBV coinfecting patients (from the former Romanian pediatric HIV cohort) liver fibrosis is associated with longer use of Lamivudine and shorter use of Tenofovir.

Keywords: HIV-HBV coinfection, liver fibrosis, Romanian HIV pediatric cohort

Abbreviations

AZT – Zidovudine	ETR – Etravirine	NNRTI– non-nucleoside reverse transcriptase inhibitor
BMI – body mass index	HbsAg– Hepatitis B virus antigen	NS – not statistically significant
CMV – Cytomegalovirus	HBV – hepatitis B virus	PCP – Pneumocystis jiroveci pneumonia
d4T – Stavudine	HCV – hepatitis C virus	Px – patients
ddC – Zalcitabine	HDV –hepatitis D virus	RTV – Ritonavir
ddI – Didanosine	HIV – human immunodeficiency virus	
EFV – Efavirenz	LF – liver fibrosis	

Corresponding author:
Giorgiana-Nicoleta Lungu
E-mail: lungugiorgiananicoleta@gmail.com

Article History:
Received: 19 February 2024
Accepted: 28 March 2024

INTRODUCTION

Liver fibrosis (LF) is the consequence of chronic liver inflammation produced by various factors that lead to the accumulation of certain proteins (mainly collagen) in the liver. Long term development of LF concludes with the occurrence of cirrhosis and liver failure which, with the exception of liver transplantation cases, can lead to the death of the patients. Latest estimations of the burden of chronic liver diseases (including liver cancers, responsible for 600,000 up to 900,000 deaths) show that the condition is responsible for one up to two million deaths annually or 2-4% of all deaths worldwide [1-3]. The most studied factors that impair liver function are chronic viral hepatitis, alcoholism and non-alcoholic hepatic steatosis.

HIV infection is also a global threat; currently there are about 39 million people infected and another 40 million lost their lives due to the infection [4]. The current estimation of chronic HBV infection is 296 million people suffering (in 2019), with 1.5 million newly infected annually [5]. The exact number of HIV-HBV coinfections is not known, but a recent review of the worldwide data estimates a prevalence of HIV-HBV coinfections of 7.6% (IQR 5.6-12.1), or 2.7 million people coinfecting, suggesting that 1 case in 100 people with HBV infection also have HIV infection as well [6].

A large number of children from Romania were infected with HIV in the past, most of them being born during 1987-1989 (the so called Romanian pediatric HIV cohort). The exact route of infection is not known, but it's considered to be iatrogenic. Also, the time of infection is considered to be their first year of life. A significant proportion of those children were also coinfecting with HBV. Today, most of the HIV infected people from our country under medical surveillance and monitoring are survivors from that cohort [7].

OBJECTIVE

To evaluate liver fibrosis in long-time monitored HIV-HBV coinfecting patients (from the former Romanian HIV pediatric cohort) using liver transient elastography-Fibro Scan.

METHODS

This is a prospective study (January 2022-June 2023) performed at the "Victor Babes" Hospital of Infectious Diseases and Pulmonology from Craiova. We have reviewed a total of 636 patients parenterally infected with HIV during early childhood, from which 183 were alive at the beginning of 2022, 271

were deceased, and 182 were lost from follow-up; from those alive, we have randomly selected a number of 37 HIV-HBV coinfecting patients to perform liver transient elastography (Fibroscan). Level of fibrosis were quantified as follows: normal value (1.6-5.5 kPa), F1 grade (7-7.1 kPa), F2 grade (7.2-9.5 kPa), F3 grade (9.6-12.5 kPa) and F4 grade or cirrhosis (>12.5 kPa). Several factors (beside chronic HBV infection) were considered risk factors for liver fibrosis and / or inflammation (body mass index – BMI) coinfections with hepatitis B, C and / or D virus, Cytomegalovirus infection, tuberculosis, antiretroviral treatments – drugs and duration –, antifungal and/or antineoplastic treatments, prophylaxis with Trimethoprim-Sulfamethoxazole, alcohol consumption, presence of dyslipidemia), introduced into a Microsoft Excel database and analyzed; every risk factor represents 1 point and a total score for risk factors were attributed to each participant; we have formed two main groups: A, with patients with HIV-HBV coinfection and liver fibrosis, and B, with those coinfecting, but without liver stiffness. Statistical comparisons were made using Chi2 with Yates' correction (two tailed) and unpaired t-test the level of statistical significance being $p < 0.05$.

Every participant signed an informed consent for participation to the present study and publication of data.

RESULTS

Group A consists of 13 patients with liver fibrosis, and group B consists of 24 individuals without liver stiffness.

General characteristics

21 patients are males (57%), most of them (19, 51.4%) live in rural settings and the majority of them (19, 51.4%) are from Dolj county. Mean age is 34.03 ± 2 years and the patients were monitored for an average of 26.11 ± 4.31 years. Average age when they were detected with HIV infection 7.91 ± 4.05 years, with no statistical difference between the two groups. Based on their clinical and immunological data they are classified (Classification of HIV infection, CDC, 1993) as: B1 = 1 (2.7%), B2 = 3 (8.11%), B3 = 16 (43.2%), C1 = 2 (5.41%), C2 = 2 (5.41%) and C3 = 13 (35.1%), also with no statistical differences between groups.

Data regarding liver transient elastography

Mean value of liver stiffness is higher in group A vs group B (11.27 ± 5.72 vs 4.93 ± 0.98 kPa, $p < 0.0001$). Data regarding liver fibrosis and grading are presented in Table 1.

TABLE 1. Data regarding liver fibrosis and grading for the considered group

Item	Group A (n=13)	Group B (n=24)
F1	1	No liver fibrosis
F2	6	
F3	4	
F4 (cirrhosis)	2	

Data regarding risk factors for liver fibrosis and/or inflammation

The average number of considered risk factors for liver fibrosis and / or inflammation is similar in both groups (8.84 ± 1.4 vs 8.33 ± 2.18 , p is not statistically significant). Details of every considered item are presented in Table 2.

TABLE 2. Risk factors for liver fibrosis and / or inflammation for the two studied groups

Risk factor	Group A (n=13) No. of Px.	Group B (n=24) No. of Px.	p value
Persisting presence of HBs Ag	13	24	0.0001
Presence of anti HDV antibodies	0	1	NS
Presence of anti HCV antibodies	0	0	–
CMV exposure	2	3	NS
Past or present tuberculosis	4	11	NS
Past or present antifungal treatment	11	21	NS
Past or present PCP prophylaxis	12	20	NS
Past or present antineoplastic therapy	1	2	NS
Past or present AZT use	13	21	NS
Past or present ddI use	6	10	NS
Past or present ddC use	8	12	NS
Past or present d4T use	9	14	NS
Past or present NNRTI (EFV, ETR) use	13	19	NS
Past or present RTV use	11	24	NS
Obesity	1	0	NS
Dyslipidemia	7	10	NS
Past or present heavy alcohol use	4	8	NS

Legend: HBs Ag = Hepatitis B virus surface antigen, HDV = hepatitis D virus, HCV = hepatitis C virus, PCP = Pneumocystis jiroveci pneumonia, AZT = Zidovudine, ddI = Didanosine, ddC = Zalcitabine, d4T = Stavudine, NNRTI = non-nucleoside reverse transcriptase inhibitor, EFV = Efavirenz, ETR = Etravirine, RTV = Ritonavir, NS = not statistically significant, Px = patients

Immunological, virological and therapeutic data

The average CD4 count is slightly higher in group A vs group B (463.71 ± 227.31 vs 450.53 ± 201.75 cells/mm³, p value is not statistically significant). Six pa-

tients from group A and 3 from group B have mean CD4 count less than 200 (p=0.06, proximal to statistical significance). Also, mean value for nadir CD4 is lower in group A compared with group B (80.46 ± 98.39 vs 137.88 ± 187.8 , p value is not statistically significant).

Average viral load for HIV is 19611.52 ± 36626.84 in group A and 42499.38 ± 139851.81 copies/mm³, but without statistically significant difference.

Duration of antiretroviral treatment (ART) is similar for both groups (263.85 ± 40.7 vs 258.54 ± 56.89 months).

Certain antiretrovirals (Lamivudine – 3TC, Emtricitabine – FTC, Tenofovir – TNF) also have active effects against HBV. Data regarding anti-HBV therapy are shown in Table 3.

TABLE 3. Duration of anti-HBV therapy in considered groups and statistical significance

Parameter	Group A (n=13)	Group B (n=24)	p value
3TC therapy (months)	227.69 ± 32.3	168.29 ± 77.17	0.01
FTC therapy (months)	4.15 ± 6.65	16.08 ± 29.73	NS
TDF therapy (months)	12.92 ± 19.88	46.92 ± 46.91	0.01
Total anti HBV therapy (months)	236.46 ± 31.13	202.21 ± 63.79	NS

Legend: 3TC = Lamivudine, FTC = Emtricitabine, TDF = Tenofovir, HBV = Hepatitis B virus

There are no statistically significant differences between group A and B regarding the duration of treatment with AZT, ddI, ddC, d4T, NNRTI or RTV, but the average length of treatment is higher for group A.

DISCUSSION

From the 636 HIV-infected patients identified with parenteral transmission during the early childhood, a number of 153 were chronically infected with HBV (only 439 were tested), that is 24.06% from the total, or 53,5% from those tested. This makes HBV infection a prime factor to be considered in relation with liver fibrosis.

There are many causes and additional risk factors for liver fibrosis in people living with HIV: demographic (older age, male gender), individual (heavy alcohol use, tobacco use, herbal medicine use, non-adherence to prescribed antiretroviral regimen), HIV-related factors (increased viral load, low CD4 cell count, use of AZT, ddI, ddC or d4T), coinfections (hepatitis B ± D, hepatitis C, schistosomiasis), metabolic (type 2 diabetes mellitus, obesity, dyslipidemia, metabolic syndrome), as well as certain ge-

netic factors [8-12]. The number of risk factors is translated into the number of liver related events and it seems that the higher the number, the higher the probability of liver fibrosis development [12]. In patients without HIV infection, metabolic factors (if steatosis is present) are more important than viral factors; also, in spite of effective antiviral therapy, progression of HBV infection might remain present at a low level and leads to liver fibrosis [13,14]. Highly effective antiretroviral therapy seems to lower the risk of liver stiffness development [15]. Our study has included some of those factors (hepatitis C or D, use of certain antiretrovirals, obesity, dyslipidemia or heavy alcohol use), but we have not found statistical differences. We have also considered other factors that might lead to liver fibrosis and/or inflammation (CMV exposure, antibiatic treatment, antifungal treatment, chemotherapy, prophylaxis with Trimethoprim/sulfamethoxazole), but we failed to notice any statistically significant differences. The number of considered risk factors are similar for both groups. While those risk factors were considered individually, it was impossible to study their overlapping and effects on liver function.

It was established that the use of Tenofovir/Emtricitabine, entry inhibitors or integrase strand transfer inhibitors are associated with better liver function; also, “older” antiretrovirals have a higher negative impact on liver function than the “newer” ones [16-19].

Our data show that for the group A (those with liver fibrosis) there is a statistically significant difference as compared with group B for the treatment with 3TC (longer in group A) and tenofovir (longer in group B). We suspect that longer use of 3TC produced the specific YMDD mutation inside HBV genotype and it would have been interesting to detect that alteration, however, we were not able to work this out for the present study.

Limitations of the study

The authors acknowledge that they have included only a small number of cases; also, the information about HBV viremic levels and resistance to certain antivirals are not available.

CONCLUSION

In this study, we conducted an evaluation of liver fibrosis using transient elastography on patients living with HIV plus HBV coinfection from the former Romanian pediatric HIV cohort due to its unique-

ness characterized by a long period of evolution of the disease, but also a long period of time under antiretroviral therapy. Because we are talking about patients born in the early 90's, they went under antiretroviral regimens with substances that nowadays are associated with liver fibrosis development. We assessed the implication of various antiretrovirals as risk factors for liver fibrosis development and we found a strong association between liver fibrosis and longer use of Lamivudine, while longer use of Tenofovir-based therapy was characteristic of patients without liver fibrosis. We also considered other risk factors like past or present various individual medical history, other coinfections, metabolic factors, however, no statistically significant differences were found between patients who developed liver fibrosis and those who did not. Liver fibrosis was identified in a significant percentage of patients living with HIV/HBV coinfection. In conclusion, this study underscores the importance of monitoring liver fibrosis in HIV/HBV coinfection for a better management of these complex patients.

Institutional Review Board Statement: This study was approved by the Committee of Ethics and Academic and Scientific Deontology of Craiova, Romania (approval no. 78/07.09.2020).

Access to the database for this study was approved by the Ethics Committee of “Victor Babes” Clinical County of Infectious Diseases and Pulmonology.

Author's contribution:

Conceptualization – Giorgiana-Nicoleta Lungu, Lucian-Ion Giubelan, Florentina Dumitrescu;
Software: Lucian-Ion Giubelan;
Methodology: Giorgiana-Nicoleta Lungu, Anca Duduvecu, Andreea Marcu;
Resources: Florentina Dumitrescu, Lucian-Ion Giubelan;
Supervision: Lucian-Ion Giubelan.

All authors have read and agreed the published version of the manuscript.

Data availability statement: The data presented in this study are available upon request from the corresponding author. The data are not publicly available due to the patients' personal data protective policy of the university and hospital.

Funding: the study received no external funding

Conflicts of interest: the authors declare no conflict of interest

There are no financial or personal relationships that might bias the content of this work.

REFERENCES

1. Devarbhavi H, Asrani SK, Arab JP, Nartey YA, Pose E, Karnath PS. Global burden of liver diseases: 2023 update. *J Hepatol.* 2023;79:516-37. doi: 10.1016/j.jhep.2023.03.017
2. Cheemerla S, Balakrishnan M. Global epidemiology of chronic liver disease. *Clin Liver Dis (Hoboken).* 2021;17(5):365-70. doi: 10.1002/cld.1061
3. Younossi ZM, Wong G, Anstee QR, Henry L. The global burden of liver disease. *Hepatology.* 2023;21(8):1978-91. doi: 10.1016/j.cgh.2023.04.015
4. World Health Organisation. HIV and AIDS (13 July 2023). www.who.int/news-room/fact-sheets/detail/hiv-aids [accessed January 2024]
5. World Health Organisation. Hepatitis B (18 July 2023). www.who.int/news-room/fact-sheets/detail/hepatitis-b [accessed January 2024]
6. Platt L, French CE, McGowan CR, Sabin K, Gower E et al. Prevalence and burden of HBV co-infection among people living with HIV: a global systematic review and meta-analysis. *J Viral Hep.* 2020;27(3):294-315. doi: 10.1111/jvh.13217
7. Compartimentul pentru monitorizarea și evaluarea infecției HIV/SIDA în România. Institutul Național de Boli Infecțioase “Prof. dr. Matei Balș”. Evoluția HIV în România update 30 iunie 2023. www.cnlas.ro/index.php/date-statistice [accessed January 2024]
8. Iacob DG, Luminos M, Benea OE, Tudor AM, Olariu CM et al. *Front Med.* 2022, doi: 10.3389/fmed.2022.888050
9. Perazzo H, Cardoso SW, Yanavich C, Nunes EP, Morata M et al. Predictive factors associated with liver fibrosis and steatosis by transient elastography in patients with HIV mono-infection under long-term combined antiretroviral therapy. *J Int AIDS Soc.* 2018. doi: 10.1002/jia2.25201
10. Wekesa C, Kirk GD, Aizire J, Benson EM, Karabinda A et al. Prevalence and factors associated with liver fibrosis among adult HIV-infected patients attending urban and rural care clinics in Uganda. *Open Forum Inf Dis.* 2020 Nov;7(11):ofaa483. doi: 10/1093/ofid/ofaa483
11. Stabinski L, Reynolds SJ, Ocama P, Laeyendecker O, Boaz I et al. High prevalence of liver fibrosis associated with HIV infection: a cross-sectional study in rural Rakai, Uganda. *Antivir Ther.* 2011;11(3):405-11. doi: 10.3851/IMP1783
12. Androutsakos T, Schina M., Pouliakis A., Kontos A, Sipsas N., Hatzis G. Causative factors of liver fibrosis in HIV-infected patients. A single center study. *BMC Gastroenterol.* 2020 Apr 6;20(1):91. doi: 10.1186/s12876-020-01230-1
13. Lv H, Jiang Y, Zhu G, Liu S, Wang D et al. *Scientific Rep.* 2023, doi: [org/10.1038/s41598-023-28351-3](https://doi.org/10.1038/s41598-023-28351-3)
14. Sun Y, Wu X, Zhou J, Meng T, Wang B et al. Persistent low level of hepatitis B virus promotes fibrosis progression during therapy. *Clin Gastroenterol Hepatol.* 2020;18:2582-91.e6. doi: 10.1016/j.cgh.2020.03.001
15. Gebremicael G, Tola HH, Gebreegziabier A, Kassa D. Incidence of hepatotoxicity and factors associated during highly active antiretroviral therapy in people living with HIV in Ethiopia: a prospective cohort study. *HIV/AIDS Res Palliative Care.* 2021;13:329-36. doi: 10.2147/HIV.S283076
16. Ezhilarasan D, Srilekha M, Raghu R. HAART and hepatotoxicity. *J Applied Pharmaceutical Sci.* 2017;7(4):220-6. doi: 10.7324/JAPS.2017.70433
17. Otto AO, Rivera CG, Zeuli JD, Temesgen Z. Hepatotoxicity of contemporary antiretroviral drugs: a review and evaluation of published clinical data. *Cells.* 2021 May 20;10(5):1263. doi: 10.3390/cells10051263
18. Mira JA, Collado A, Rivero-Juarez A, Monje-Agudo P, Ruiz-Morales J et al. Liver toxicity of current antiretroviral regimens in HIV-infected patients with chronic viral hepatitis in a real-life settings: a HEPAVIR SEG-HEP cohort. *Plos ONE.* 2016;11(2):e0148104. doi: 10.1371/journal.pone.0148104
19. Rivera CG, Otto AO, Zeuli JD, Temesgen Z. Hepatotoxicity of contemporary antiretroviral drugs. *Curr Op HIV AIDS.* 2021;16(6):279-85. doi: 10.1097/COH.0000000000000706