

Prevalence and Factors Associated with HIV and Hepatitis B and Hepatitis C Co-Infection in Children Attended at the Hubert Koutoukou Maga National University Teaching Hospital (CNHU-HKM) of Cotonou

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Abstract

Background: Viral hepatitis B and C share the same transmissions route with HIV. This fact could explain the relative high prevalence of HIV and Hepatitis B and C virus co-infection.

Objective: The purpose of this study is to determine HIV and Hepatitis B and C virus co-infection frequency among HIV infected children's cohort at Cotonou National Teaching Hospital and identify predicting factors of this co-infection.

Materials and methods: Authors performed a descriptive, cross-sectionnal and analytic study covering the period of 1st of May to 31st of August at the Cotonou National Teaching Hospital which is a tertiary hospital dedicated to HIV infected children follow up and management. Recruitment was exhaustive and sociodemographic, clinical and biological data (Ag HBs, ac HbC) were registered.

Results: 31 cases of co-infection were registered among 234 HIV infected children (13.2%). HIV/VHB co-infection was encountered in 12.8% of cases and HIV/VHC co-infection in 0.4%. Through univariate analysis history of blood transfusion was an associated factor and through multivariate analysis, predicting factors of that co-infection were length of HAART ($p=0.0375$), children's hepatitis B immunization status (0.0461) and history of blood transfusion ($p=0.0162$).

Conclusion: This work will contribute to reinforce regular screening of hepatitis B and C co-infection among HIV infected children before HAART initiation and also serve as tool for advocacy for hepatitis B immunization at birth in our country.

Keywords: Prevalence; Hepatitis B; Hepatitis C; Seric markers; HIV; Co-infection

Introduction

Mortality associated with opportunistic infections due to human immunodeficiency virus (HIV) has significantly decreased over the last two decades, because of successful highly active antiretroviral therapies. However, chronic liver diseases are increasingly recognized as a leading cause of morbidity and mortality in patients with HIV and hepatitis B virus (HBV) or hepatitis C virus (HCV) co-infection [1,2].

Hepatotropic virus has the same routes of transmission as HIV, thus the existence of a high frequency of infection by the viruses of hepatitis B and C in HIV-positive patients [3]. The negative effect of HIV infection on the outcome of hepatitis B and C is well established. HIV infection worsens prognosis for liver diseases associated with HCV and HBV [2]. More specifically, HIV facilitates transition to chronicity and fast progression toward liver fibrosis and hepatocellular carcinoma (HCC) [4-6]. Viral hepatitis would increase the risk for HIV transmission from mother to child and HIV viremia. It was also noted in some co-infected patients a fast progression of HIV infection and a reduction in response to antiretroviral therapy.

It is estimated that 10% of people living with HIV worldwide are co-infected with hepatitis B virus; this rate may reach 25% in the endemic areas [7]. HBV/HCV comorbidity testing in people living with HIV is therefore an important systematic screening tool. In Benin, most studies conducted on those co-infections focused on adults [3,4]. In 2014, a study carried out at the Borgou Regional University Teaching Hospital in Parakou by d'Almeida et al. on HIV/HBV co-infection has noted a high seroprevalence estimated at 9.62% in the HIV infected children's cohort [8]. We thought it was a good idea to determine the prevalence and factors associated with HIV co-infection and viral hepatitis B and C among HIV- infected children attended in the pediatric referral unit of CNHU/HKM in Cotonou. Those results may be used as the basis for advocacy with the authorities in charge of HIV disease control in order to implement systematic free of charge co-infection screening.

Patients and Methods

Type and period of study

It was a cross-sectional, descriptive and analytical study carried out from the 1st of June to the 31st of August 2015 at the CNHU/HKM of

Cotonou which is the referral site dedicated to pediatric care for HIV infection. The study was performed in the pediatric unit for the selection of children, in the laboratories of the Outpatient Treatment Centre (OTC) providing care to patients living with HIV and in the Blood Bank for the biological test processing.

Study target population

The study population consisted of all the HIV-infected children attended for care and follow up at the CNHU/HKM pediatric unit.

Inclusion and exclusion criteria

The survey involved HIV-infected children aged 0 to 15 years at the time of testing, attended in the pediatric unit, who benefitted from screening for HBV and HCV and whose parents gave their written consent.

The infants exposed to HIV whose viral or serological status according to age was not yet identified, were not included.

Sampling and recruitment

Sampling was exhaustive, since it has taken into account all the children meeting the inclusion criteria.

Data collection

The data were gathered using a data collection form on the basis of children's clinical records, completed by an interview of parents or guardians. Then, the biological data were completed as soon as results were available.

Variables

The variables collected were related to the sociodemographic characteristics (age, sex, educational background, vital status and socioeconomic status of parents), clinical (HBV immunization status, history of blood transfusion, circumcision or excision, piercing, scarification, sexual abuse, sexual activity, children's WHO clinical stage during the study period, presence of hepatomegaly and jaundice), biological (biological markers of hepatitis B and C, alanine transaminases/ALT, CD4 cell count, viral load) and therapeutical characteristics (Duration of antiretroviral therapy, line of therapy and treatment) of children and parents.

Operational procedures

In practical terms, all the children whose parents gave their written consent benefitted from complete physical examination. At the end of consultation, 5ml of venous blood was collected into an evacuated dry tube and an EDTA tube for the estimation of CD4 cell count and viral load during the survey. The code written on the questionnaire was reported on the tubes. The labeled blood samples were immediately carried to the OTC laboratory and to the CNHU blood bank for processing. Once in laboratory, the samples were immediately centrifuged, decanted, and aliquots were formed with transcription of the code. The serums thus obtained were stored at -20°C until analysis of the serum markers of hepatitis B and C. The technique used was immunological and enzymatic. The references of the reagents used were: Monolisa HBs Ag Ultra ≠ 5C0081; Monolisa HBe Ag-Ab Plus ≠

4H0040; Monolisa anti-HBc Plus ≠ 5C0091; Monolisa anti-HBc IgM Plus ≠ 4L0090; - Reagent Monolisa HCV Ag-Ab Ultra ≠ 5C0018.

Determination of HBV infection: HBsAg testing was systematic. In case they proved positive, the tests were continued so as to determine HBeAg, HBeAc and HBcAc. In the case of HIV-negative status for HBsAg, the tests were continued in order to identify total anti-HBc antibodies. All the positive tests were systematically checked again to ensure that results are accurate.

Determination of HCV infection: Testing for anti-HCV antibodies was also systematic and all positive samples were checked again. The reagent used was Monolisa HVC Ag-Ab Ultra ≠ 5C0018.

Transaminase testing: Transaminases were performed with spectrophotometer using EliTech reagent ≠ 14-2770. Erba Norm serum was used for quality control.

Estimation of CD4 cell count: Venous samples were processed at the laboratory of HIV infection Outpatient Treatment Centre (OTC/CTA) located inside the hospital. The technique used was centrifugation by flow cytometry (FCM). Immunological status was assessed through CD4 cell count. Thus, CD4 cell count <100 cells/mm³ has been considered as a severe immunosuppression, and a rate between 100 and 350 cells/mm³ regarded as moderate immunosuppression. CD4 cell count more than 350 cells/mm³ means normal immunological status i.e., no immunosuppression.

Data processing

The software tools Epi Data 3 and STATA/IC 11.0 were used for data entry and analysis. Ratios were compared by means of Chi² or Fisher test. Logistic regression with univariate or multivariate analysis enabled to measure the relationship. Significance threshold adopted was 5%.

Ethical considerations: Families received detailed information about the course of the study. Their consent was required before any inclusion. As regards children with age above 12 years, an individual interview was done to explain the course of the study. Parents were informed on therapeutic possibilities in case of co-infection.

Results

Prevalence of co-infection: Out of the 234 children involved in the study, 31 had co-infection i.e., an overall frequency of 13.24%. Concerning HIV and hepatitis B co-infection, that prevalence was estimated at 12.82% i.e., respectively a 4.27% seroprevalence for HBsAg and an 8.55% isolated seroprevalence for HBe Ac. HIV and hepatitis C co-infection was estimated at 0.42% whereas triple seroprevalence of HIV and hepatitis B and C co-infection was estimated at 0.42%. The serological profile of HIV/HBV co-infected patients is specified in Table 1.

Socio-demographic characteristics of the study target population

The mean age of co-infected patients was 10 ± 4.2 years without significant difference with HIV non-coinfected patients. Table 2 summarizes sociodemographic characteristics of the children involved in the study.

	HBsAg	Total HBcAc	HBc IgM Ac	HBeAg	HBeAc	Total
Inactive/convalescent carrier	+	+	-	-	+	3
Possible reactivation	+	+	-	-	-	4
Acute hepatitis	+	+	+	-	+	1
Chronic hepatitis in replication	+	+	-	+	-	2
Occult Infection	-	+	-	-	-	20

Table 1: Serological profile and stages of infection of HBV/HIV co-infected patients.

	Co-infection n (%)		Total (n=234)	p1
	Yes (n=31)	No (n=203)		
Sex				0.847
Female	15 (48)	102(50)	117 (50)	
Male	16 (52)	101 (50)	117 (50)	
Age				0.514
<5	04 (13)	46 (23)	50 (21)	
05 to 10	12 (39)	67 (33)	79 (34)	
11 to 15	13 (42)	68 (33)	81 (35)	
15 to 17	02 (6)	22 (11)	24 (10)	
Educational background				0.36
Uneducated	04 (13)	35 (17)	39 (17)	
Primary school	21 (68)	108 (53)	129 (55)	
Secondary school	06 (19)	60 (30)	66 (28)	
Parents' vital status				0.244
Alive	16 (52)	127 (63)	143 (61)	
Deceased	15 (48)	76 (37)	91 (39)	
Socioeconomic status				0.376
Low*	21 (68)	108 (54)	129 (55)	
Average**	09 (29)	82 (40)	91 (39)	
High***	01 (3)	13 (6)	14 (6)	

‡ Chi² test to compare the sociodemographic characteristics of co-infected patients versus patients with one infection; * <50; ** between 50 and 100; *** >100 USD.

Table 2: Distribution of patients according to their socio-demographic characteristics.

Clinical history of the study target population

Hepatitis B immunization's history was found among 65% of the co-infected children whereas history of jaundice was found in 16%, blood transfusion's history among 55% of them. Circumcision's history was found in 42% of targeted children and sexual activity found in 3%. Table 3 shows the detailed distribution of children according to their medical history.

Biological characteristics of the study target population

Among the 31 co-infected children, 14 (45%) had detectable viral load, and 17 (55%) had moderate to severe immunodeficiency. Table 4 recaps the distribution of children according to their immunological and viral status and transaminases values.

	Co-infection (n (%))		Total (n=234)	p
	Yes (n=31)	No (n=203)		
Hepatitis B immunization				0.067
Yes	11 (35)	42 (21)	53 (23)	
No	20 (65)	161 (79)	181 (77)	
Jaundice				0.11
Yes	5 (16)	12 (6)	17 (7)	
No	26 (12)	191 (94)	217 (93)	
Blood transfusion				0.011
Yes	17 (55)	64 (32)	81 (35)	
No	14 (45)	139 (68)	153 (65)	
Injection				0.91
Yes	22 (71)	141 (69)	163 (70)	
No	9 (29)	62 (31)	71 (30)	
Piercing				0.847
Yes	15 (48)	102 (50)	117 (50)	
No	16 (52)	101 (50)	117 (50)	
Circumcision				0.898
Yes	13 (42)	81 (40)	94 (40)	
No	18 (58)	122 (60)	140 (60)	
Scarification				0.569
Yes	12 (39)	68 (33)	80 (34)	
No	19 (61)	135 (67)	154 (66)	
Sexual activity				0.465
Yes	01 (3)	12 (6)	13 (6)	
No	30 (97)	191 (94)	221 (94)	

*Chi square test was used for comparison of clinical history between co- infected and HIV infected children.

Table 3: Distribution of HIV and VHB/VHC co-infected children according to their clinical history.

	Co-infection n (%)		Total	p
	Yes (n=31)	No (n=203)		
Immunological status				0.577
Normal	14 (45)	111 (55)	125 (53)	
Moderate deficiency	12 (39)	69 (34)	81 (35)	
Severe deficiency	05 (16)	23 (11)	28 (12)	
Viral load				0.348

Detectable	14 (45)	110 (54)	124 (53)	
Undetectable	17 (55)	93 (46)	110 (47)	
ALT				0.239
Normal	28 (90)	193 (95)	221 (94)	
Abnormal	3 (10)	10 (5)	13 (06)	
Duration of ART				0.088
No ART	01 (03)	16 (08)	17 (07)	
<1year	07 (23)	40 (20)	47 (20)	
1-5 years	17 (55)	70 (34)	87 (37)	
≥ 5 years	06 (19)	77 (38)	83 (36)	
Line of treatment				0.131
1st*	26 (87)	142 (76)	168 (77)	
2nd**	04 (13)	46 (24)	50 (23)	
1st line treatment	n=30	n=188	n=218	0.645
2NRTI+ 1 NNRTI	24 (80)	155 (82)	179 (82)	
2 NRTI+ 1IP	06 (20)	28 (15)	34 (16)	
3 INRT	00 (00)	05 (03)	05 (02)	
Chi-square test to compare the therapeutical characteristics of co-infected versus mono-infected patients.				
*In 1st line, all the treatments were based on Lamivudine.				
**In 2nd line, only two of the co-infected patients were on a treatment based on Lamivudine. No one of them was on tenofovir disoproxil fumarate (TDF).				
NRTI: Nucleosidic Revesre Transcriptase Inhibitors; NNRTI: Non Nucleosidic Reverse Transcriptase Inhibitors; IP: Protease Inhibitors.				

Table 4: Distribution of children according to their biological characteristics at inclusion in study and therapies.

Therapeutic characteristics (ART) of the study target population

Almost all the co-infected patients were on antiretroviral therapy. The duration of treatment was comprised between 1 and 5 years in 55% of them. Table 4 shows the distribution of population according to ART.

Factors associated with co-infection

After multivariate analysis through logistic regression, three factors were predictive of significant risk for co-infection among the study population: history of blood transfusion, immunization status and duration of antiretroviral therapy (ART). Table 5 shows the predictors of co-infection and relationships observed.

	cOR	aOR	CI‡ (95%)	p†
Blood Transfusion				0.0162
No	1	1	1	
Yes	2.64	2.64	(1.20-5.85)	
Length of HAART				0.0375
No HAART	1	1	1	
<1 year	2.8	2.01	(0.22-18.32)	
1-5 years	3.89	2.88	(0.35-23.85)	
≥ 5 years	1.25	0.71	(0.75-6.73)	

Immunization status against hepatitis B virus				0.0461
Accurate	1	1	1	
Inaccurate	0.47	0.4	(0.17-0.97)	
cOR: Crude Odds Ratio; aOR: Adjusted Odds Ratio for blood transfusion, duration of treatment, immunization status; ‡CI (95%) Confidence interval at 95%; †p; p value of Wald's Chi-square.				

Table 5: Predictors of co-infection.

Discussion

This study has enabled to determine the prevalence of HIV and hepatitis B/C co-infection among the cohort of children living with HIV attended at the CNHU of Cotonou. The prevalence of HIV/HBV co-infection is 12.39%, which is close to the one found out at national level among blood donors in 2012 [3]. That rate is lower than the 16.9% reported in the adults in 2012 [4] but higher than the 9.62% reported in children of North-Benin in 2015 [8]. In Ethiopia, Abera et al. had identified a significantly low rate of 2% [5]. In Nigeria, Emeka et al. had reported a rate of HBV/HIV co-infection of 5.8% [6]. Other authors working on cohorts of children had also reported rates lower than ours, particularly Zhou et al. in China in 2010 with 4.9% and Telatela et al. in Tanzania with 1.2% [9,10]. The high prevalence noted in our research work may be due to the fact that Benin is classified among the countries with high prevalence >8% [11]. Moreover, in this study all the children with hepatitis B core antibody (HBc Ac) were considered as HBV co-infected whereas the studies mentioned above took into account only presence of hepatitis B surface antigen (HBsAg). Hepatitis B core antibody (HBcAc) is the first antibody which appeared during HBV infection and is permanently persistent [12]. It is associated with HBsAg during the acute and chronic phase of infection, and with hepatitis B surface antibody (HBsAc) in case of recovery. The interpretation of that <isolated HBcAc> profile is difficult for it may correspond to different situations that are pathological or not: acute, chronic and occult infection or post-infectious immunity according to clinical and biological facts reported by Pol [13]. In Mexico, Alvarez-Muñoz et al. in a study conducted among people living with HIV (PLHIV) with negative HBsAg had found out 49% of occult infection after viral DNA testing [14]. In the United Kingdom, Chadwick et al. had found 4.5% of occult infection in African migrants living with HIV and with negative HBsAg profile [15]. We think that it is relevant, in our areas where most children are infected before 5 years of age, to consider <isolated HBcAc> profile as a factor that may reflect the existence of occult infection. In this research work, HIV/HCV co-infection is low (0.4%). This result is close to the 0.46% rate reported by Kerubo et al. in Kenya, and to the 0.7% rate reported by Chiekulie et al. in Nigeria among adults [16,17]. Abera et al. had reported a higher rate of 5.5% [5]. Only one patient involved in the study presented with a triple co-infection. This result is similar to the one reported by other authors, thus confirming the scarcity of triple co-infection [16-18].

The mean age of co-infected children was 10 ± 4.2 years, without significant difference from the one of children with one infection (9.53 ± 4.4 years). It is close to the one found out by several authors; this suggests a perinatal infection or an infection in early childhood of hepatitis among those children [5,6]. As the risk for transition from chronicity to HBV infection is higher in case of infection in early childhood, it is necessary to ensure prevention through immunization

at birth in countries with high prevalence [19]. Most children involved in the survey had a normal rate of transaminases, but 45% of them had a detectable viral load, and 55% an immunodeficiency, without significant relationship with co-infection. In Nigeria, Olawumi et al. had found that HIV/HBV co-infection was associated with a decline in CD4 cell count, and an increase in transaminases [20]. Transaminases increase has also been reported by Pol [13]. Abera et al. had also identified a relationship between co-infection and elevated transaminases [5]. On the contrary, Baseke et al. in Uganda, had not identified any disorder of transaminases in co-infected patients [18]. As a result, co-infection impact on CD4 and liver enzymes varies depending on studies, and the effect of hepatotoxic drugs is not yet clearly established [13,18]. Almost all the children involved in the study were on ART, and 80% on first-line treatment including lamivudine. That molecule has an excellent action on HBV and HIV, but is a low genetic barrier with rapid occurrence of resistance [13,21]. An assessment of compliance and even of resistance tests would be necessary in co-infected children with detectable viral load, in order to ensure a possible readjustment of their treatment. The factors associated with co-infection in this study were history of blood transfusion, lack of immunization against hepatitis B, and duration of ART lower than five years. Other authors have also identified blood transfusion as associated with co-infection in Benin and Nigeria [22,23]. We can assume with the results in Table 5 that HIV infected children with history of blood transfusion are running 2.64 times risk of hepatitis. Though we are in a context that blood was tested for hepatitis B and C before being collected in the blood banks. Safe blood transfusion issue in the emergent countries remains a relevant one. On the contrary, we cannot assume an association between length of HAART and co-infection although $p=0.0375$, based on the CI95% values (0.22-18.32) for instance. Immunization status against HBV ($p=0.0461$) seems to be a protecting factor in co-infected children.

Conclusion

HIV and hepatitis co-infection is a reality among children at the National University Teaching University (CNHU); this exposes them to severe liver failure complications. Therefore, there is an urgent need to put in place in Benin a systematic immunization of newborns at birth against hepatitis B, as well as a systematic screening before putting them on antiretroviral (ARV) drugs.

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