

# HIV co-infection with hepatitis B and C viruses among Nigerian children in an antiretroviral treatment programme

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**Background.** Nigeria has one of the world's largest burdens of children living with HIV and is highly endemic for hepatitis B. This study set out to determine the prevalence of hepatitis B and C infections among HIV-infected children and to identify the factors associated with these co-infections.

**Method.** We studied 155 HIV-infected children. Information on socio-demographics and history of exposure to risk factors such as scarification, blood transfusion, unsafe injections and circumcision were obtained. All the children were tested for the presence of hepatitis B surface antigen and antibodies to hepatitis C.

**Result.** The prevalence of HIV/HBV co-infection was 7.7%, while that of HIV/HCV co-infection was 5.2%. No child was co-infected with all three viruses. Children who were co-infected with HCV were more likely to be older than 5 years. There was no significant association between co-infection with either of the hepatitis viruses and socio-economic status, gender, number of persons living in the household, World Health Organization clinical stage, route of acquisition of HIV, scarification, blood transfusion, unsafe injection or circumcision.

**Conclusion.** The rate of HIV co-infection with hepatitis B and C in children is significant. HIV-infected children should be screened for these viruses. Those found to be negative and not immunised for hepatitis B should be immunised. Since the natural history of these co-infections in children is not known, it is imperative that affected patients be followed up adequately.

With a national seroprevalence of 4.6% and a population of 148 million, Nigeria has a very large burden of people living with HIV/AIDS (PLWHA).<sup>1,2</sup> Nigeria reportedly has the highest burden of mother-to-child transmission in the world, and has over 240 000 children living with HIV/AIDS, representing 15% of the African burden.<sup>3</sup> Nigeria is also endemic for hepatitis B virus (HBV) infection,<sup>4</sup> a virus that shares similar transmission routes with HIV.<sup>5</sup> Hepatitis C virus (HCV)<sup>6</sup> has also been reported in the general Nigerian population.<sup>7</sup> HIV co-infections with HBV and HCV have been documented in adults<sup>8-12</sup> and children,<sup>6,13,14</sup> but there is a paucity of data on this subject among children in resource-limited settings.

The population prevalence of HIV/HBV co-infection in Africa is thought to reflect the population prevalence of hepatitis B surface antigen (HBsAg).<sup>5</sup> Several studies in Nigerian children have recorded prevalence rates of HBsAg ranging from 7.5% to 44.7%, varying from one locale to another.<sup>15,16</sup> The prevalence of HBsAg among children in our area was 10.8%.<sup>17</sup> It is therefore expected that the prevalence of HIV/HBV co-infection will vary from locale to locale. The prevalences of HIV/HBV in children from Tanzania and Cote d'Ivoire have been reported as 1.2% and 12.1%, respectively,<sup>13,14</sup> while the prevalence of HIV/HBV co-infection among Nigerian children was 8.3%.<sup>18</sup>

It is believed that in Africa most hepatitis B infection is acquired horizontally, with the infection being transmitted from child to child before the age of 5 years.<sup>4</sup> This is in contrast to Asia, where vertical

transmission is more prominent because of the higher prevalence of hepatitis e antigen.<sup>4</sup> It is possible that in Africa higher HIV/HBV co-infection may be present, as immunosuppression caused by HIV infection acquired vertically may predispose the child to acquiring hepatitis B infection horizontally.

There are few studies on the prevalence of hepatitis C in the general population of Nigerian children. A study published in 1996<sup>19</sup> reported a 0% prevalence among preschool children, while another study in Benin City<sup>20</sup> reported a prevalence of 0.25%. The rate of HIV/HCV co-infection in Tanzanian children was 13.8%.<sup>13</sup> No child was found to be co-infected with HCV in an Ivorian study.<sup>14</sup>

In Nigeria some of the blood that is transfused is not routinely screened for hepatitis C,<sup>8</sup> so with many children requiring transfusion for severe anaemia from various causes (notably malaria), the prevalence of hepatitis C may be significant.<sup>21</sup> This would be in addition to vertically acquired HCV, which is said to be more likely if the mother is also HIV infected and has a high HCV viral load.<sup>22-24</sup> In a Nigerian study on co-infection in children, HIV/HCV co-infection was recorded in 2.7% of the children studied.<sup>18</sup> This study did not have controls and did not explore possible risk factors.

Co-infection with all three organisms has also been recorded. In Nigerian adults the prevalence was 7.2% in Keffi<sup>10</sup> and 1% in Ibadan,<sup>25</sup> while in the study on Nigerian children co-infection with all three viruses was reported in only one child in the entire cohort studied.<sup>18</sup>

Both hepatitis B and C viruses are known to lead to chronic infections after the acute infection.<sup>4,26</sup> HBV infections acquired in the perinatal period and early childhood are more likely to lead to chronic infection.<sup>4</sup> Hepatitis C, on the other hand, is likely to lead to chronic infection in up to 60 - 80% of patients after an acute infection.<sup>26</sup> With improved survival of HIV-infected patients due to the effect of antiretroviral therapy (ART), it is reported that liver disease (resulting from chronic infection with hepatitis B and C) has become an important cause of morbidity and mortality among these patients in developed countries.<sup>27,28</sup> Progression to liver disease has also been reported to be faster in co-infected persons.<sup>29</sup> It is not known whether this is also the case in children.

Most studies on co-infection in Nigeria have been in adults. With increasing access to ART in Nigeria, it is expected that more HIV-infected and co-infected children will survive. It is therefore important that the burden of these co-infections be quantified and the risk factors for co-infection in the large population of Nigerian children infected with HIV identified.

This study was carried out to determine the prevalence of co-infections with hepatitis B and C viruses in HIV-infected Nigerian children and to identify socio-demographic factors associated with the co-infections.

## Methodology

The study was carried out in the paediatric HIV/AIDS clinics of the University of Benin Teaching Hospital between October and December 2009. The paediatric HIV/AIDS clinic is one of the President's Emergency Plan Funds for AIDS Relief (PEPFAR) sites, where antiretrovirals are provided free. The attendees at the clinic receive general paediatric and specialist care as required. Their CD4 counts and packed cell volumes are monitored on a 3-monthly basis, while liver function tests and electrolytes, urea and creatinine are monitored biannually. CD4 percentage is monitored only in children younger than 5 years. Hepatitis B and C are not routinely screened for at enrolment into the treatment programme.

Consecutive children aged 2 months to 17 years who were confirmed to be HIV infected by enzyme-linked immunosorbent assay (ELISA) in those older than 18 months or by DNA polymerase chain reaction (PCR) if younger than 18 months were enrolled. Verbal consent was obtained from parents/guardians, and from children older than 10 years.

A sample of 150 was recruited, having calculated the sample size using the formula

$$n = \frac{z^2 pq}{d^2}$$

where n = sample size, z = standard deviation (1.96), p = prevalence, q = 1-p and d = degrees of freedom (0.05), and assuming a prevalence of 10.8% based on the most recent hepatitis B surface antigen prevalence for the study setting.<sup>17</sup>

Information on demographics was obtained from parents/guardians, using a pro-forma. Information included age, gender, ethnic group, living conditions (number of members in the family, number of rooms), parental educational level and occupation. Socio-economic class was determined using the method described by Olusanya *et al.*<sup>30</sup> Possible risk factors for acquisition of the infections such as scarification, blood transfusion, unsafe injections and surgery were ascertained. The hepatitis B vaccination status of each child was also ascertained.

The World Health Organization clinical stage of the subjects at enrolment into the programme was obtained from their medical records.

## Laboratory examination

All study subjects were screened for HBsAg, antibody to hepatitis C virus (anti-HCV) and antibody to the hepatitis B core antigen (anti-HBc) by ELISA using the respective AUTOBIO diagnostic kits. All assay protocols, cut-offs and interpretation were according to the manufacturer's instructions.

## Statistical analysis

All data were entered into a Microsoft Excel spreadsheet. Analysis was done using Statistical Package for Social Sciences (SPSS) version 13 and Graphpad InStat 3. Prevalences were recorded as simple percentages. Means were computed and comparisons between means were done using one-way analysis of variance (ANOVA) and Student's *t*-test as appropriate. Associations between non-parametric variables were tested using Fishers's exact test. The level of significance was set at 0.05.

## Results

We recruited 155 HIV-infected children for the study. There were 91 males and 64 females, giving a male/female ratio of 1.42:1. The mean age (standard deviation (SD)) of the children was 6.76 (SD 3.8) years, with a range of 10 months - 17 years. Of the 155 children 12 (7.7%) were positive for HBsAg and 8 (5.2%) positive for anti-HBc. The prevalence of past and present exposure to hepatitis B was therefore 12.9%. The prevalence of anti-HCV among the children was 5.2%. No child was co-infected with all three viruses.

The demographic features of the HIV mono-infected and HIV/ HBV and HIV/HCV co-infected children are shown in Table I. The mean ages of those with HIV mono-infection, HBV co-infection and HCV co-infection were 6.69 (SD 3.75), 6.99 (SD 4.82) and 7.48 (SD 3.91) years, respectively. These differences were not significant (*p*=0.83). However, the children co-infected with HCV were more likely to be older than 5 years compared with those co-infected with HBV (7 of 8 (87.5%) v. 6 of 12 (50.0%)), odds ratio 7.00, confidence interval 0.65 - 75.78).

Table II shows the associations of hepatitis B and C co-infection with some risk factors for horizontal transmission. No significant associations were found.

Hepatitis B vaccine only became widely available in Nigeria in 2004. Assuming that any child older than 5 years was unlikely to have received hepatitis B immunisation, we therefore analysed only the hepatitis B immunisation history of the under-5s. Of the 59 under-5s, 5 (8.5%) reportedly had incomplete or no immunisation for hepatitis B. Of the 6 under-5s who were co-infected with HBV, 5 (83.3%) reportedly had complete hepatitis B immunisation.

## Discussion

The mean age of the children in our study was higher than that in other studies from Nigeria or Cote d'Ivoire.<sup>18,14</sup> This may be because the children in those studies were evaluated at enrolment into the treatment programme, while our study was a cross-sectional study of children already in the treatment programme.

The prevalence of hepatitis B in the studied children is lower than the 10.8% recorded in a study on non-HIV-infected children from the study setting some 19 years earlier.<sup>17</sup> This lower prevalence refutes the hypothesis that hepatitis B infection may be higher in HIV-positive children. It is, however, pertinent to note that hepatitis B immunisation was introduced to the country in the time interval between the two studies. This may have masked a possibly expected higher prevalence of hepatitis B.

It would have been expected that the under-5s in this study should have benefited from hepatitis B immunisation, resulting in a lower

TABLE I. CHARACTERISTICS OF CHILDREN WITH HIV MONO-INFECTION AND THOSE WITH CO-INFECTION

Characteristic	HIV only (N=135)	HIV/HBV (N=12)	HIV/HCV (N=8)	Total	p-value
Gender (N(%))					
Female	55 (40.7)	5 (41.7)	4 (50.0)	64	
Male	80 (59.3)	7 (58.3)	4 (50.0)	91	0.81
Mean age (yrs) (SD)	6.69 (3.75)	6.99 (4.82)	7.48 (3.91)		0.83
Route of acquisition of HIV (N(%))					
Vertical	111 (82.2)	9 (75.0)	7 (87.5)	127	
Infected blood	9 (6.7)	2 (16.7)	0 (0.0)	11	0.76
Sexual	2 (1.5)	0 (0.0)	0 (0.0)	2	
Unknown	13 (9.6)	1 (8.3)	1 (12.5)	15	
Socio-economic status (N(%))					
High	25 (26.0)	1 (16.7)	0 (0.0)	26	
Middle	17 (17.7)	1 (16.7)	0 (0.0)	18	0.30
Low	54 (56.3)	4 (66.7)	3 (100.0)	61	
No. in household (N(%))					
≤4	67 (50.4)	4 (33.3)	3 (37.5)	74	0.24
≥5	66 (49.6)	8 (66.7)	5 (62.5)	79	
WHO clinical stage at entry* (N(%))					
Stage I (A1 & P1)	34 (25.2)	4 (33.3)	1 (12.5)	39	
Stage II (A2 & P2)	37 (27.4)	2 (16.7)	2 (25.0)	41	0.63
Stage III (A3 & P3)	58 (43.0)	4 (33.3)	5 (62.5)	67	
Stage IV (P4)	6 (4.4)	2 (16.7)	0 (0.0)	8	

\*A1, A2 and A3 are WHO clinical stages for adolescents, while P1, P2, P3 and P4 are the stages for paediatric patients.

TABLE II. ASSOCIATION OF HEPATITIS B AND C CO-INFECTIONS WITH RISK FACTORS FOR HORIZONTAL TRANSMISSION

Risk factor	Co-infected	Mono-infected	p-value
Circumcision (N(%))			
Yes	13 (65.0)	98 (73.1)	0.44
No	7 (35.0)	36 (26.9)	
Ear piercing (N(%))			
Yes	9 (45.0)	61 (45.2)	1.00
No	11 (55.0)	74 (54.8)	
Unsafe injections (N(%))			
Yes	2 (10.5)	22 (16.4)	0.74
No	17 (89.5)	112 (83.6)	
Blood transfusion (N(%))			
Yes	4 (22.2)	49 (37.4)	0.30
No	14 (77.8)	82 (62.6)	
Surgery (N(%))			
Yes	1 (5.0)	17 (12.7)	0.4
No	19 (95.0)	117 (87.3)	

prevalence of hepatitis B in this age group. This was not the case, as the age-specific prevalence of hepatitis B was not significantly different from other groups. Some of the children who were reported to have received the vaccine actually tested positive for HBsAg. The finding of HbsAg positivity among immunised children has been documented previously.<sup>31</sup> It is possible that these children may not have been immunised, had been incompletely immunised or were cases of vaccine failure. It was not possible to verify claims of immunisation in these children.

Although the mean ages of children with HIV/HBV, HIV/HCV and HIV only were not different statistically (as was also found in the Nigerian study),<sup>18</sup> the children who were co-infected with hepatitis

C tended to be older than those who were co-infected with hepatitis B. The two infections share similar transmission routes, but hepatitis B is also transmitted horizontally, especially among young children.

As has been documented from previous studies in Nigeria, circumcision, ear piercing, parenteral injections and blood transfusion were not associated with seropositivity for HBsAg in this study.<sup>32,33</sup> These findings are similar to those from Tanzania but differ from those in Pakistan, in which an association was found between HBsAg positivity and receipt of therapeutic injections as well as increasing numbers of injections.<sup>13,34</sup> The Pakistani study, however, also found no associations between HBsAg seroprevalence and ear piercing and tattooing. The lack of association between some of these practices, which have potential for transmission of hepatitis B and C and HIV, may be associated with the current drive for high awareness of HIV, which has increased knowledge of prevention methods, including the observance of universal precautions. In one study, for example, it was reported that traditional healers now request new blades from their clients for procedures such as scarifications.<sup>32</sup>

Some 5% of the studied children tested positive for antibodies to hepatitis C. This prevalence is almost twice that in Ibadan in Nigeria (2.7%), but is considerably lower than that in Tanzania (13.8%).<sup>18</sup> In Cote d'Ivoire there was no co-infection with hepatitis C.<sup>14</sup> This shows that the prevalence of hepatitis C co-infection with HIV varies considerably from locale to locale within the sub-Saharan region. The prevalence in this study was also higher than that reported in an American study.<sup>35</sup> Comprehensive screening of blood donors and expanded use of highly active antiretroviral therapy (HAART) were the reasons suggested for the low prevalence of hepatitis C among the younger HIV-infected children in that study.

Horizontal transmission is not documented for hepatitis C. In Tanzania under-5s were found to have a higher incidence of HCV/HIV co-infection, and the authors suggested that this may be related to vertical acquisition of HCV.<sup>13</sup> It would have been expected that if the infection was transmitted vertically more under-5s in our study would have had the infection, but this was not so, suggesting other possible routes of acquisition of HCV among these children. This may reflect differing modes of transmission of HCV that may be specific to our setting. Lending credence to this hypothesis is the low

prevalence (1.86%) of HCV among antenatal attendees in the same setting,<sup>36</sup> where no mother was dually infected with HIV and HCV. Blood transfusion, which is a major route of transmission of hepatitis C, was not significantly associated with hepatitis C infection, in contrast to a Chinese study.<sup>6</sup> Other procedures where contaminated sharps may be used, such as scarification, circumcision, ear piercing, unsafe injections and surgery, were also not significantly associated with hepatitis C in this study. These findings are similar to those in Pakistan and Tanzania.<sup>34,13</sup>

This study has shown that the burden of co-infections of hepatitis B and C viruses with HIV is significant. While not identifying any risk factors for co-infection with these organisms, it has drawn attention to the lack of full understanding of the epidemiology of these viral co-infections among children. More research is needed to define locale-specific epidemiology and to study the natural history of co-infections in children. The first step towards these research endeavours is identification of those who are co-infected. All children already in the programme and new entrants should be screened for both HBV and HCV, so that those who are co-infected can be identified for specific follow-up.

A large proportion of HIV-infected children are not co-infected with HBV. Most older children are likely not to be immunised against HBV. This group of children will remain at risk for hepatitis B because of the high burden of the disease in the general population.

A reasonable approach will be to immunise all children born before 2004 (when HBV vaccine became available through the national programme on immunisation) and who are not infected or immune, on entry to an HIV treatment programme. Obviously the routine national hepatitis B immunisation programme will also need to be strengthened.

**Conflict of interest:** None declared.

**Author contribution:** AES: conceptualised the work, was involved in data collection and wrote the manuscript. WES: contributed to the final concept of the work, analysed the data and reviewed the final draft. NJJ: contributed to the final concept of the work, was involved in data collection, entered the data and reviewed the final draft.

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