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Hepatitis B and C Co-Infections in HIV-Infected Children: Nutritional and Immune Status Assessment

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Abstract

Co-infection with hepatitis B and/or C virus (HBV or HCV) in HIV infected children is of increasing clinical importance. Children under the age of 15 years make up about 10% of the total HIV-positive population and at least 90% live in Sub-Saharan Africa. This study aim to determine the Nutritional and Immunological Status of Hepatitis B and/Or Hepatitis C Infection in HIV Infected Children. The study was done at the Irrua Specialist Teaching Hospital (ISTH), Irrua, and Edo State. A total of 86 HIV positive respondents were therefore recruited as Subjects and 86 HIV negative respondents recruited as Controls. One hundred and eighty-three respondents, 90 Subjects and 93 Controls, were initially recruited. However, the blood samples of 4 Subjects and 7 Controls were haemolysed and thus unsuitable for analysis. Thus, a total of 172 respondents, 86 Subjects and 86 Controls were finally included in the analysis. The age range of the Subjects was 0.5 - 15 years and that of the Control 0.8 – 15 years. The mean ± SD age of the Subjects (7.2 ± 4.4 years) was not significantly different from that of the Controls (6.4 ± 4.1 years; t = 1.208, p = 0.229). General weakness was the commonest symptom (present in 10.5% of Subjects and 4.7% of Controls) and hepatomegaly the commonest physical sign (present in 30.2% of Subjects and 10.5% of Controls). Fever was present in 8.1% of the Controls and none of the Subjects. The prevalence of HBV and/or HCV co-infection in HIV-infected children is not associated with the clinical stage of HIV infection or the degree of immune suppression as measured with CD4+ count or CD4 percent. The prevalence of HBV and/or HCV co-infection in HIV-infected children is reflective of the prevalence of HBV and HCV infections in the general childhood

population. Co-infection of HBV together with HCV is rare in HIV-infected children, as it is in the general population of children. The prevalence of HBV and HCV co-infections in HIV-infected children is high. Therefore, screening for these co-infections should be part of the standard programme of management of HIV-infected children.

Keywords: Nutrition; Immunology; Hepatitis B; Hepatitis C; HIV; Children

Introduction

Co-infection with hepatitis B and/or C virus (HBV or HCV) in HIV infected children is of increasing clinical importance [1-3]. This is because all three viruses are associated with devastating infections in their own individual capacities and the combined infections could be additive in their effect(s) [4,5]. For example, HBV co-infection increases the risk of cirrhosis, and is associated with higher levels of HBV replication, lower rates of spontaneous resolution of the HBV infection, and a higher risk of reactivation of previous infections [5, 6]. HCV accelerates the evolution and progression of liver disease in HIV-infected individuals [7, 8] and both HBV and HCV infections also increase the toxicity to antiretroviral medications [4]. The prevalence of HBV co-infection in Africa is thought to reflect the population prevalence of hepatitis B surface antigen (HBsAg) [9]. Studies in the general population of Nigerian children report HBsAg prevalence rates of 7.5% to 44.7%, depending on the locale [10-13] while the prevalence of HBV co-infection in HIV infected Nigerian children is 8.3% [14]. Studies from other African countries report HBsAg prevalence of 1.2% to 12.1% in the general population of children [12,15] and HBV co-infection prevalence of 13.8% in HIV infected children.

Children under the age of 15 years make up about 10% of the total HIV-positive population and at least 90% live in Sub-Saharan Africa. Thus, HIV/AIDS is a major cause of infant and childhood mortality in Africa. In addition, in children under five years of age, HIV/AIDS now accounts for 7.7% of mortality worldwide and is responsible for > 19% rise in infant mortality and a 36% rise in under five mortalities. Together with factors such as declining immunization, HIV/AIDS is a major threat to the previous gains in infant and child survival and health. The clinical course of paediatric HIV infection is more rapid than that in adults. Also, mortality in HIV infected African children is higher than in their counterparts in developed countries [16]. This may be due to the higher prevalence of malnutrition, limited access to care and treatment including access to ART and, above all, high rates of inter-current infections including infection with the hepatitis viruses among African children [16,17]. The pathogenesis of HIV disease is complex and multi-factorial and a significant part of the pathophysiology derives from the associated immunologic dysfunction [18]. A wide array of immune system deficits is associated with HIV infection and abnormalities in the function of all limbs of the immune system, including T- and B-lymphocytes, antigen-presenting cells, natural killer cells, and neutrophils have been described. These three viruses share similar or common routes of transmission [2,20]. This is mostly through blood and blood products, sexual contact and vertically from mother to newborns. High-risk populations include patients receiving multiple blood transfusions, patients on haemodialysis, and those with multiple sexual partners [21]. Vertical transmission of HCV is seen in less than 6% of neonates. Therefore, this study aim to determine the Nutritional and Immunological Status of Hepatitis B and/Or Hepatitis C Infection in HIV Infected Children.

Materials and Method

Study Area

The study was done at the Irrua Specialist Teaching Hospital (ISTH), Irrua, and Edo State. Irrua is a rural community in Esan Central Local Government Area (LGA), in the Central Senatorial District of Edo State in the South-South Geopolitical Zone of Nigeria. The Hospital is located about 87 kilometres from Benin City, the State Capital, alongside the Abuja-Benin Expressway. Esan Central LGA has an estimated population of 128,571 and covers a land area of 436 square kilometers [22]. ISTH serves as a tertiary care referral hospital to other hospitals and health centres in Edo Central and North Senatorial Districts, and the neighboring states of Ondo, Kogi, and Delta States. It is a 375-bed Hospital and an average of 7,815 and 1,015 children are seen in the Paediatric Out-Patient Clinic and the Children's Emergency Room, respectively, per year. ISTH has a Paediatric Antiretroviral (ART) Clinic, which is supported by the Institute of Human Virology, Nigeria (IHVN). Antiretrovirals (ARVs) are provided free in the Clinic, which is attended by a weekly average of 10 patients. The attendees at the Clinic receive general paediatric and specialist care as required and their CD4+ counts are monitored on a 3-monthly basis. CD4+ percentage is calculated for children younger than 5 years. There is no standard programme of screening for HCV, as this is not funded for in the support from IHVN.

Study Design

This was a prospective, observational, case-control, cross-sectional study.

Study Population

The Subjects and Controls, aged six months – 15 years, were recruited at the Paediatric Out- Patients Clinic (POPC), the

Children's Emergency Room (CHER) and Paediatric ART Clinic of ISTH, Irrua.

Selection Criteria

Inclusion criteria: Consecutive HIV-positive children, irrespective of the WHO Clinical Stage, whose parents/legal guardians gave written informed consent were recruited as Subjects based on the under-listed criteria:

- Age not less than six months and not greater than 15 years.
- HIV-positive on being screened as part of the Provider Initiated Testing and Counseling (PITC) programme.
- HIV positive children referred from other health facilities.
- HIV-positive children of patients attending the Adult HIV Clinic of ISTH; and
- Symptomatic infants of HIV-positive mothers who are older than 6 months and are HIV DNA PCR positive.

Exclusion criteria

- Age <6 months; and
- Refusal of parents/guardians to give written informed consent

Controls

The Controls were recruited from among HIV negative children attending the POPC or admitted at the CHER based on the criteria:

Inclusion Criteria: Age 6 months – 15 years; and HIV infection status re-confirmed as negative on further testing;

Exclusion Criteria: Age < 6 months or >15 years, Refusal of parents/guardians to give written informed consent; and Non-confirmation of HIV negative status on retesting.

Sample Size Estimation

The minimum sample size for the study was estimated using the formula for comparison of two study populations, in this instance HIV infected children (Subjects) and non-HIV infected children (Controls), n = pqz^2, where n = minimum sample size, p = prevalence, q = 1- p, z = $d2$ normal standard deviation for the required level of confidence usually set at 1.96, and d = tolerable margin of error usually set at 5% [23]. Substituting the 15.0% combined prevalence of HBV and HCV co-infection in HIV infected adults reported from a case-control study in Lagos [24],

$$n = \frac{0.150 \times 0.850 \times 1.96^2}{0.05^2} = 195.9$$

However, allowing for the fact that the sample population is <10,000 because the total number of children registered in the HIV/AIDS programme in ISTH at the time of the study was 130, and applying the correction factor [27] Nf = n/1 + (n/N) and substituting,

Nf = 195.9/1 + (195.9/130) = 195.9/1 + 1.5069 = 195.9/2.5069 = 78.14,

Thus giving 78 as the minimum sample size, which the addition of 10% as attrition for non- responders made 85.95 or 86 as the final sample size. A total of 86 HIV positive respondents were therefore recruited as Subjects and 86 HIV negative respondents recruited as Controls.

Method of Collection of Data

Interviews were conducted using a structured questionnaire to obtain information on demographic characteristics, immunization history, presence of risk factors for HBV or HCV infection such as scarification of the skin, blood transfusion, unsafe injections and surgery, circumcision and the presence of clinical features of hepatitis in the preceding 6 months before recruitment into the study. The socio-economic class of the families of the respondent was determined using the method described by Agelebe et al., [25]. In this method, socioeconomic status is determined by finding the average of the educational attainment and occupation of the mother and the father. The mean of four scores (two for the father and two for the mother) to the nearest whole number is the social class assigned to the respondent. The socioeconomic classes are then classified into upper (classes I to III) and lower (classes IV and V).

Clinical Evaluation

Each child underwent a full physical examination by the Researcher to determine the presence or absence of the clinical features described in the questionnaire and had samples taken for the determination of HBsAg, anti-HCV, CD4+T-Lymphocyte counts and full blood count (FBC) of the Subjects and Controls. The clinical stage of HIV infection in the Subjects was determined using the updated World Health Organization's Revised Human Immunodeficiency Virus Pediatric Classification System.

Laboratory Evaluation

Sample collection

Five millilitres of blood was drawn aseptically by venepuncture from the forearm and transferred into a labelled plastic microlitre tube containing ethylene diamine tetraacetic acid (EDTA) as anticoagulant. Two milliliters of blood was set aside for the full blood count and CD4+ analysis. The supernatant obtained after centrifugation was carefully decanted into a new tube and coded to ensure confidentiality and stored at -200 C until analyzed. The sera were analyzed in the IHVN Laboratory for HIV (Controls

only), Hepatitis B surface antigen (HbsAg), antibodies to Hepatitis C (anti-HCV) and full blood count.

Analysis of samples

Analysis of the blood samples was done by two dedicated Laboratory Scientists in the IHVN Laboratory, ISTH within 1 hour of collection of the samples. The Researcher observed this process in the Laboratory.

HIV Test: The screening for HIV was done using Determine HIV-1/2 (Abbott Laboratories, Illinois, USA) and Unigold HIV-1/HIV-2 (Trinity Biotech PLC, Jamestown, New York, USA) for antibodies to HIV I and II. This is a rapid immunochromatographic method for the quantitative detection of antibodies of all isotopes (IgG, IgM, IgA) specific to HIV-1 and HIV-2 simultaneously in serum. A sample was considered HIV antibody-positive if the serum is reactive to both tests. Where discordance occurred, HIV 1/2 STATPAK rapid test kit (Chembio Diagnostics Systems, Inc., Medford, NY 11763, USA) was used as a tie-breaker according to the WHO double /triple algorithm.

HBsAg Test: Serum for serological assays for HBsAg was stored at −20°C until the time for assay. The testing for HBV infection was done using ACON Hepatitis B surface antigen rapid test strip (Acon Laboratories Inc., San Diego, CA, USA).

Anti-HCV Test: Serum for serological assays for anti-HCV was stored at −20°C until the time for assay. Antibodies were detected using a commercial third generation qualitative ELISA test (MONOLISA anti-HCV plus version 2, Biorad, Marnes-La-Coquette, France).

Determination of Full Blood Count: This was also done in the IHVN Laboratory of ISTH, using a Sysmex KX-2IN automated analyzer manufactured by Microfield Instrument England. The machine quantifies total and differential blood counts by automation. The determination of full blood count was done within 1 hour of venepuncture.

T-Lymphocyte CD4 + Count: CD4+T lymphocyte count was determined by flow cytometry using Becton Dickson Facs calibur machine (Partec, Germany). The CD4+ T lymphocyte count was performed within three hours of sample collection. Twenty µl of the patient's blood was incubated with a fluorescent CD4 monoclonal antibody (Partec CD4 easy count kit), which recognizes the T lymphocytes CD4 surface antigen. After 15 minutes, the reaction was stopped and the sample was run in the flow cytometer, which gives the CD4 count in cells/µl of blood.CD4 percent was calculated for children <years of age by first obtaining the lymphocyte fraction from the full blood count result. The lymphocyte fraction was then divided by 100 and multiplied by the total white blood cell count to derive the Total Lymphocyte Count. The CD4+ count divided by the Total Lymphocyte Count is then multiplied by 100 to give the CD4 percent. Thus, (Lymphocyte Fraction/100) × Total White Blood Cell Count = Total Lymphocyte Count and (CD4 count/ Total lymphocyte count) × 100 = CD4 percent [17, 28].

Data Analysis

Data was analyzed with International Business Machines Corporation Statistical Product and Service Solution (IBM SPSS) Version 20.0 [26] for sorting, calculation of means and standard deviations. Pearson's chi-square ($\chi 2$) test was used to determine the significance of the difference between frequencies. However, where the assumptions for $\chi 2$ could not be met in a 2 x 2 table, Fisher's exact test was used instead. Student's t-test and one-way analysis of variance (ANOVA) were used as appropriate to determine the association between continuous variables. The level of statistical significance was set at $p < 0.05$ in all the analyses.

Results

One hundred and eighty-three respondents, 90 Subjects and 93 Controls, were initially recruited. However, the blood samples of 4 Subjects and 7 Controls were haemolysed and thus unsuitable for analysis. Thus, a total of 172 respondents, 86 Subjects and 86 Controls were finally included in the analysis.

Sociodemographic and Clinical and Laboratory Characteristics of the Subjects and Controls

The age range of the Subjects was 0.5 - 15 years and that of the Control 0.8 – 15 years. The mean ± SD age of the Subjects (7.2 ± 4.4 years) was not significantly different from that of the Controls (6.4 ± 4.1 years; t = 1.208, p = 0.229). The age, gender, socioeconomic class and ethnic group distribution of the Subjects and Controls is shown in Table I. Thirty-eight (44.2%) Subjects and 48 (55.8%) Controls were ≤5 years old, and 45 (52.3%) versus 50 (58.1%) were males. There was no statistically significant difference between the number of Subjects and Controls aged <5 years (p = 0.127). There was also no significant difference between them in the number of males (p = 0.440). Thirty-four (39.5%) Subjects and 15 (17.5%) Controls were from the higher socioeconomic class (SEC) families and 52 (60.5%) versus 57 (66.3) of the Esan ethnic group (Table 1). The number of Subjects from the higher SEC was significantly higher than that of the Controls (p = 0.001). The difference in the number drawn from the Esan ethnic group was not significant (p = 0.565).

Prevalence of symptoms and signs of hepatitis, and prevalence of risk factors for HBV or HCV infection, in Subjects and Controls

The prevalence of symptoms and signs of hepatitis in the Subjects and Controls at recruitment is shown in (Table 2). General weakness was the commonest symptom (present in 10.5% of

Subjects and 4.7% of Controls) and hepatomegaly the commonest physical sign (present in 30.2% of Subjects and 10.5% of Controls). Fever was present in 8.1% of the Controls and none of the Subjects. The difference between the Subjects and Controls in the prevalence of fever (p = 0.007), hepatomegaly (p <0.001) and splenomegaly (p = 0.028) was statistically significant. The differences in the prevalence of the other features were not significant. The prevalence of risk factors for infection with HBV or HCV in the Subjects and Controls is shown in (Table 3). A history of circumcision by unqualified persons or in unsterile settings, was the most common risk factor in both Subjects (57.0%) and Controls (58.1%). Making traditional scarifications marks on the skin (46.5% versus 14.0%), sharing of hair clippers (44.2% versus 48.8%), and a past history of blood transfusion (27.9% versus 4.7%) were the next three most frequent risk factors. The Subjects had a significantly higher prevalence of a history blood transfusion (p <0.001), scarification marks (p <0.001) and the sharing of unsafe injection needles (p = 0.009). There were no significant differences in the prevalence of the other risk factors.

***Table 1**: Age, Gender, Socioeconomic Status and Ethnic Groups of Subjects and Controls.*

Variable	Status	Subjects	Controls	χ^2	*P*
		N = 86	**N = 86**		
Age	≤5 years	38 (44.2)	48 (55.8)	2.33	0.127
	>5 Years	48 (55.8)	38 (44.2)		
Gender	Male	45 (52.3)	50 (58.1)	0.59	0.44
	Female	41 (47.7)	36 (41.9)		
M:F ratio	-	1.1:1	1.3:1		
Socioeconomic Status	Upper classes	34 (39.5)	15 (17.5)	10.30	0.001
	Lower classes	52 (60.5)	71 (82.5)		
Ethnicity	Esan	52 (60.5)	57 (66.3)		
	Non-Esan Edo	23 (26.7)	22 (25.6)	1.14	0.565
	Other groups	11 (12.7)	7 (8.1)		

Note: Percentages in brackets and add downwards. NA = not applicable.

***Table 2**: Prevalence of Symptoms and Signs of Hepatitis in Subjects and Controls*

Clinical feature	Subjects	Controls	χ^2	*P*
	N = 86	**N = 86**		
Fever	0 (0.0)	7 (8.1)	7.30	0.007
Passage of coke coloured urine	1 (1.2)	2 (2.3)	0.34	0.56
Weakness	9 (10.5)	4 (4.7)	2.08	0.149
Itching	5 (5.8)	3 (3.5)	1.51	0.471
Jaundice	1 (1.2)	1 (1.2)	1.01	0.605
Hepatomegaly	26 (30.2)	9 (10.5)	10.37	<0.001
Splenomegaly	17 (19.8)	7 (8.1)	4.84	0.028

Note: Percentages in brackets and read across.

***Table 3**: Prevalence of Risk Factors for Infection with HBV or HCV in Subjects and Controls*

Risk Factors	Subjects (N =86)	Controls (N=86)	χ^2	*p*
Blood transfusion	24 (27.9)	4 (4.7)	17.06	<0.001
Incomplete HBV vaccination	1 (1.2)	3 (3.5)	2.00	0.368
Hepatitis diagnosed in relative	0 (0.0)	1 (1.2)	2.00	0.368
Sharing of unsafe injection needles	11 (12.8)	2 (2.3)	6.74	0.009
Sharing of hair clippers	38 (44.2)	42 (48.8)	0.37	0.541
Traditional uvulectomy	1 (1.2)	0 (0.0)	1.01	0.316
Past history of dental surgery	3 (3.5)	4 (4.7)	0.15	0.700

Circumcision	49 (57.0)	50 (58.1)	0.02	0.877
Past history of surgery	4 (4.7)	2 (2.3)	0.69	0.406
Scarification marks	40 (46.5)	12 (14.0)	21.61	<0.001
Tattoo marks	4 (4.7)	9 (10.5)	2.08	0.149

Note: Percentages in brackets and read across.

***Table 4**: Mean ± SD Weight, Height and Body Mass Index of Subjects and Controls.*

	Subjects	Controls	t	*p*
Mean ± SD weight in Kg	19.5 ± 8.5	20.0 ± 17.1	-0.219	0.827
Mean ± SD height in cm	111.7 ± 25.4	107.8 ± 23.7	1.024	0.307
Mean ± SD BMI	26.7 ± 4.6	27.7 ± 3.2	1.58	0.115

***Table 5**: Mean ± SD Packed Cell Volume, and Mean ± SD Total and Differential White Blood Cell Counts of Subjects and Controls.*

	Subjects	Controls	t	*P*
Mean ± SD PCV in %	32.2 ± 3.2	39.7 ± 42.8	-1.631	0.105
Mean ± SD WBC/mm^2	6665.1 ± 2538.1	7377.9 ± 2842.4	-1.735	0.085
Mean ± SD % neutrophils	32.8 ± 9.3	39.9 ± 14.0	-3.921	<0.001
Mean ± SD % lymphocytes	56.4 ± 9.8	51.6 ± 14.8	2.509	0.013

***Table 6**: Mean ± SD CD4+ T-Lymphocyte Count and Mean (SD) CD4 Percent of Subjects and Controls*

	N	Subjects	N	Controls	t	*P*
CD4+ count/micro L	48	1146.78 ±551.3	38	1318.2 ± 634.05	-1.340	0.184
CD4 percent	38	26.3 ± 13.2	48	46.7 ± 70.6	-1.96	0.053

***Table 7**: Mean ± SD Weight, Height and Body Mass Index of HBV or HCV Infected and Non-Infected Subjects and Controls*

Variable	Infection status*	Subjects		Controls		t/*p*
		N	Mean ± SD	N	Mean ± SD	
Weight in kg	+ve	13	19.2 ± 9.2	8	15.7 ± 10.5	0.80/0.432
	-ve	73	19.6 ± 8.4	78	20.4 ± 17.6	0.35/0.723
t/*p*			**0.17/0.867**		**0.75/0.456**	
Height in cm	+ve	13	116.5 ± 28.0	8	88.4 ± 19.9	2.47/0.023
	-ve	73	110.8 ± 25	78	109.8 ± 23.3	0.25/0.800
t/*p*			**0.74/0.464**		**2.51/0.014**	
BMI	+ve	13	14.1 ± 3.7	8	23.4 ± 25.0	1.34/0.196
	-ve	73	25.4 ± 21.1	78	26.6 ± 22.8	
t/*p*			**1.91/0.059**		**0.38/0.704**	

**+ve = infected with hepatitis B or C viruses, -ve = not infected with hepatitis B or C viruses. BMI = body mass index.*

***Table 8**: Mean ± SD Packed Cell Volume and White Blood Cell Count of HBV Or HCV Infected and Non-Infected Subjects and Controls*

Variable	Infection status*		Subjects		Controls		t/*p*
			N	Mean ± SD	N	Mean ± SD	
PCV in %	+ve		13	33.2 ± 2.2	8	36.0 ± 2.4	2.74/0.013
	-ve		73	31.9 ± 3.4	78	40.1 ± 4.9	11.87<0.001
t/*p*				**1.30/0.200**		**2.34/0.022**	
Total WBC	+ve		13	7261.5 ± 3608.7	8	8300.0 ± 2567.9	0.71/0.488
	-ve		73	6558.9 ± 2315.0	78	7283.3 ±2867.4	1.70/0.091
t/*p*				**0.92/0.361**		**0.96/0.338**	
% Neutrophils	+ve		13	35.7 ± 5.6	8	33.6 ± 17.5	1.70/0.091

	-ve		73	32.3 ± 9.7	78	40.5 ± 13.5	4.26/<0.001
t/*p*				**1.22/0.225**		**1.34/0.183**	
% Lymphocytes	+ve		13	51.6 ± 8.6	8	53.4 ± 18.0	0.31/0.759
	-ve		73	57.3 ± 9.8	78	51.4 ± 14.6	2.90/0.004
t/*p*				**1.98/0.051**		**0.36/0.720**	

**+ve = infected with hepatitis B or C viruses, -ve = not infected with hepatitis B or C viruses. PCV = packed cell volume; WBC = white blood cell count.*

Growth and Nutritional Status of Subjects and Controls

The growth status of Subjects and Controls in terms of their mean ± SD height and weight is shown in (Table 4). The Controls were heavier by about 0.45 kg and shorter by about 4 cm but the differences were not statistically significant (p = 0.827 for the difference in mean weight and p = 0.307 for the difference in mean height).

Mean ± SD Packed Cell Volume and White Blood Cell Counts of Subjects and Controls

The mean ± SD packed cell volume (PCV), and mean ± SD total and differential white blood cell (WBC) counts of the Subjects and Controls is shown in Table V and the mean ± SD CD4+ T lymphocyte count and mean ± SD CD4 percent in Table VI. The mean PCV and mean total WBC count of the Controls were higher by 7.5% and 712.8 WBC/mm3, respectively. However, the differences did not attain statistical significance (p = 0.105 for the difference in mean PCV and p = 0.085 for the difference in mean WBC count). The mean percentage neutrophil count of the Controls was higher by 7.1% while the mean percentage lymphocyte count of the Subjects was higher by 4.8% (Table 5). The differences were highly significant (p <0.001 for the difference in percentage neutrophil count and p = 0.013 for the difference in percentage lymphocyte count).The mean CD4+ count and mean CD4 percent of the Controls were higher by 171.4/mm3 and 20.4%, respectively (Table 6). However, the difference in mean CD4+ count and mean CD4 percent did not attain statistical significance (p = 0.184 for the difference in mean CD4+ count and p = 0.053 for the difference in mean CD4 percent).

Mean ± SD of Growth and Nutrition Indices Associated with HBV and HCV Infections in Subjects and Controls

This is shown in Table VII. The mean weight of infected Subjects was higher than that of the infected Controls by 3.5 kg and the height by 28.1 cm while the mean BMI was lower by 9.3. The difference in mean ± SD height between the infected Subjects and infected Controls was statistically significant (p = 0.023) but the other differences were not (p = 0.456 for the difference in mean weight, p = 0.196 for the difference in mean BMI). There were no significant differences between uninfected Subjects and uninfected Controls. There were also no significant differences between infected and uninfected Subjects in their mean weight (p = 0.867) and mean height (p = 0.464) but the difference in mean BMI approached statistical significance (p = 0.059) (Table 7). Infected Controls had a significantly lower mean height than uninfected Controls (p = 0.014). The other differences between infected and uninfected Controls were not statistically significant (p = 0.456 for the difference in mean weight, p = 0.704 for the difference in mean BMI).

Mean ± SD packed cell volume and white blood cell count of HBV and HCV infected and uninfected Subjects and Controls

Table VIII shows the mean ± SD packed cell volume, and total and differential white blood cell count of the Subjects and Controls in relation to HBV and HCV infection status. The mean packed cell volume of infected Subjects was lower than that of the Controls by 2.8%, the mean total WBC was lower by 1038.5/mm3 and the mean percentage lymphocyte count by 1.8% but the mean percentage neutrophil count of infected Subjects was higher by 2.1%. The difference in mean packed cell volume was significant (p = 0.013) but the other differences were not statistically significant (p = 0.488 for total WBC count, 0.689 for percentage neutrophils and 0.759 for percentage lymphocytes). The mean packed cell volume (p <0.001) and mean percentage neutrophils count (p<0.001) of uninfected Subjects were significantly lower than that of uninfected Controls but the latter had a significantly lower mean percentage lymphocyte count (p = 0.004). Uninfected Controls also had a higher mean total WBC count than the uninfected Subjects, but the difference did not attain statistical significance (p = 0.091). None of the differences between infected and uninfected Subjects were significant although the lower mean percentage lymphocyte count in infected Subjects approached statistical significance (p = 0.051). A lower mean packed cell volume was the only significant difference (p = 0.022) between infected and uninfected Controls (Table 8).

Discussion

The prevalence of individual and combined infections in HIV-infected children in this study was HBV 10.5%, HCV 4.7% and HBV/HCV 0.0%. The corresponding figures in HIV- uninfected children are prevalence of HBV 5.8%, prevalence of HCV 3.5% and prevalence of HBV/HCV 0.0%. The prevalence of HBV infection in HIV-infected children is higher than the 1.2% reported from a rural population of HIV-infected children in Tanzania, [15] the 4.9% reported from Chinese children [29] and the 7.7% reported recently from nearby Benin City, Nigeria [30]. It is, however, lower than the 12.1% reported from Cote d'Ivoire [12]. This is in keeping with the known regional and intra-regional variations in the prevalence of HBV infection. The prevalence of HBV infection in HIV-infected children in this study is also higher than the 4.4% prevalence reported in HIV-infected adults from the same centre as that of the present study about two years ago [31]. The higher prevalence in the children in this study perhaps supports the hypothesis that hepatitis B infection may be higher in HIV-infected children than in HIV-infected adults because of the higher rates of horizontal transmission of HBV in sub- Saharan Africa. However, both the prevalence in children and adults in Irrua are lower than the 20.6% reported from HIV-infected adults in Jos, North Central Nigeria using similar methods. The generally higher prevalence of HIV-infection in the North Central States of Nigeria may be a factor [32]. It is also possible, however, that the methods used in the studies from Irrua may have underestimated the prevalence of co-infection with HBV in HIV-infected patients. This is because the definition of HBV infection was based on a single positive HBsAg test result as opposed to a more robust definition using sequential HBsAg results, HBV DNA testing, hepatitis B core antibodies, or a combination of these tests [33-36].

There are only few studies available on the prevalence of hepatitis C in the general population of Nigerian children. One study [37] reported a 0.0% prevalence in preschool children, while another study in Benin City, Nigeria [30] reported a prevalence of 0.25%. Horizontal transmission has not been documented in hepatitis C infections. An earlier study using plasma HCV RNA quantification in HIV-infected patients in Northern Nigeria, reported HCV co-infection prevalence of 8.2% [38]. On the other hand, no co-infection with HCV was reported from Ilorin [39]. The results of the current study is thus within the reported prevalence range of HCV co- infection in HIV patients which varies considerably from locale to locale even within the sub region. The 4.7% prevalence of HCV in HIV-infected children in the study is comparable to the 3.5% prevalence in HIV-uninfected children. The difference is unlike the much higher prevalence of HBV in HIV-infected children compared to HIV-uninfected children. Bearing in mind the general similarities in the routes of transmission of HIV, HBV and HCV, the difference in the pattern of HIV/HBV and HIV/HCV co-infections as regards the difference between HIV- infected and uninfected children in the prevalence of HBV and HCV infections is difficult to explain. The higher HCV prevalence of 3.5% in HIV-uninfected children in this study is also difficult to explain. The recent study reported from Benin City did not include HIV-uninfected children. An earlier study from Benin City [30] as discussed earlier reported an HCV prevalence of 0.25% in the general population of children. Improvements in the sensitivity and specificity of diagnostic tests may be a factor. The prevalence of HBV/ HCV co-infections in HIV-infected and uninfected children in this study is 0.0%. This is in keeping with the prevalence 0.0% - 0.4% in earlier reports in HIV- infected children from developing countries, [12,15] including Nigeria [30]. It is also in keeping with the low prevalence reported from HIV-infected Zambian adults and from blood donors in Ilorin, Nigeria [40]. However, higher prevalence rates of HBV/HCV infections have also been reported from HIV-infected adults in Nigeria. These include reports of 1.5% prevalence from Abuja [24] and 7.25% from Jos [38]. The similarities and differences in the prevalence of HBV/HCV infections between the findings in this and other studies accord with the variability of the prevalence of co- infections with HBV and HCV in HIV-infected persons worldwide, depending on the geographic region, risk group and the type of exposure involved which varies between countries and also even within the same country [20,41]. Varying sample size, test kit sensitivity and specificity may also be a factor in the variations. This may make comparisons across studies misleading because of the differences in HCV detection techniques [20,41]. The use of quantitative plasma HCV RNA is possible only in patients with active hepatitis replication [42,43]. On the other hand, anti-HCV antibodies can be detected in patients with a previous exposure, including those with ongoing viral replication and those whose immune response may have curtailed viral replication. However, there may also be rare cases of falsely negative anti-HCV antibodies in patients with advanced immunosuppression [44-46]. Other findings in this study should be discussed because of their clinical and public health importance. All the HIV-infected children with HCV co-infection were older than 5 years of age. A majority of those with HBV co-infection were also above 5 years of age. These observations are at par with those in a recent report from Benin-City, Nigeria on HIV-infected children and may be due to the impact of Hepatitis B vaccination, which was introduced into the National Programme of Immunization in 2004 [47] and was received by all the under-5s in this study. In contrast, under-5s in the Tanzanian study had a higher incidence of HCV co-infection, and the authors suggested that this may be related to vertical acquisition of HCV. It would have been expected that if the infection was transmitted vertically, more

under-5s in this and the earlier study from Benin City would have been infected. This was not so, suggesting that other routes of acquisition of HCV may be more dominant among these children.

The proportion of HIV-infected and uninfected children from families in the lower socio- economic classes was high in this study. This is in keeping with the rural/sub-urban background of the study population, in which the occupation of a majority of the persons is low scale agrarian. The mean packed cell volume of HIV-uninfected children was higher than that of HIV- infected children in the study. Anaemia in HIV infected children is due to a number of factors, which include immunological suppression, impaired erythropoietin production, blood loss from intestinal opportunistic diseases, micronutrient deficiencies, and concurrent co-morbidities like tuberculosis. The mean white blood cell count was also higher in HIV-uninfected children. This maybe as a result of the immunosuppression associated with HIV infection [48].

Conclusion

This study emphasizes the intricate relationship that exists between HIV-infected children's clinical outcomes, immunological indicators, and dietary status. The results show that immunosuppression and malnutrition continue to be key obstacles that have a big impact on clinical staging and disease progression. The importance of proper nutrition in reducing HIV-related morbidity was highlighted by the higher likelihood of advanced HIV infection and severe immune suppression in children with low nutritional indices.

Additionally, the study clearly links immunological impairment, dietary deficits, and co-infections with hepatitis, highlighting the necessity of integrated care approaches. Improving clinical outcomes in this susceptible group requires early discovery, regular nutritional evaluation, and focused interventions including dietary supplements and timely co-infection therapy.

In addition to improving immunological recovery, strengthening pediatric HIV programs to incorporate nutrition-focused tactics in addition to antiretroviral medication will lower disease burden and mortality. To improve long-term results, policymakers and healthcare professionals must give top priority to comprehensive management plans that meet the nutritional and immunological needs of children infected with HIV.

Ethical Consideration

Ethical clearance for the study was obtained from the Research and Ethics Committee of ISTH. Two trained study staff approached potential candidates, described the study procedures to the parents/legal guardian, and obtained written informed consent from the parents/guardians before enrollment into the study.

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Conflict of Interest

None to declare.

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Availability of Data and Materials

The authors declare consent for all available data present in this study.

Authors' Contributions

The entire study procedure was conducted with the involvement of all writers.

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