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RESEARCH ARTICLE

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Association Between Hepatitis B and C Co-Infection, HIV Disease Stage, and Immune Status in Pediatric Patients

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ABSTRACT

HIV infection in children is of significant public health importance because of its impact on childhood morbidity and mortality and its attendant negative impact on health services delivery. This study aims to determine the Hepatitis B and C Infection and Clinical Staging of HIV Infected Children and the Prevalence of Immunologic Suppression. 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. 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. 28% Subjects were ≤5 years old. 5 (13.2%) were in clinical stage 1, 15.8% in stage 2, 44.7% in stage 3 and 26.3% in stage 4. 55.3% had no immune suppression while 7 (18.4%) had moderate and 26.3% severe immune-suppression. 2.1% were in clinical stage 1, 31.3% in stage 2, 39.6% in stage 3 and 27.1% in stage 4. 52.1% had no immune suppression while 39.6% had moderate and 8.3% severe immune suppression. 71.1% of those under 5 years old versus 66.7% older Subjects were in clinical stages 3 and 4. 44.7% versus 47.9% had moderate – severe immune suppression while among those with immune suppression, 10/17 (58.8%) versus 4/23 (17.4%) had severe immune suppression. The prevalence of infection in this study was higher in female children with HIV infection. The prevalence of HBV/HCV co-infections in HIV-infected and uninfected children in this study is 0.0%.

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Introduction

HIV infection in children is of significant public health importance because of its impact on childhood morbidity and mortality and its attendant negative impact on health services delivery [1]. About 2.1 million children <15 years old are estimated to be living with Human Immunodeficiency Virus (HIV) infection acquired largely through mother to child transmission [2]. The problem of HIV/AIDS and of paediatric HIV/AIDS in children in Nigeria is no less serious. HIV prevalence rose from 1.8% in 1992 to a peak of 5.8% in 2001, but although the prevalence is declining, the rate of decline in no way matches that of the rate of increase [1]. Co-infection with hepatitis B and/or C virus (HBV or HCV) in HIV infected children is of increasing clinical importance [3-5]. 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) [6,7]. 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 [7,8]. HCV accelerates the evolution and progression of liver disease in HIV-infected individuals [9,10] and both HBV and HCV infections also increase the toxicity to antiretroviral medications [6].

Most of the studies on HIV/HBV and HIV/HCV co-infections in Nigeria been limited to the urban centres in the South West and North Central geopolitical zones of the country and the adult populations [11-17]. There is a dearth of information from the other geopolitical zones as well as children. This is despite

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the fact that HIV infected children are also at increased risks of co-infection with HBV and HCV owing to the prevalence of these infections in the country. In addition, little is known about the relationship between the level of immune suppression as determined by the CD4+ count/percent and the prevalence of HBV and HCV co-infections. These concerns informed the need for this study which investigated the seroprevalence of HBV and HCV co-infections and the associated CD4+ count/percent among HIV-infected children on Antiretroviral Therapy (ART) in a tertiary health institution located in a rural/semi-urban setting in Nigeria. Immunological staging for children is also possible. The absolute CD4 count and the percentage values in healthy infants who are not infected with HIV are considerably higher than those observed in uninfected adults, and slowly decline to adult values by the age of 6 years [18,19]. Either the total lymphocyte cell counts or absolute CD4+ count can be used in older children but the CD4+ count is preferred. Currently, therefore, the measurement of the CD4 percentage is recommended in younger children less than 5 years old [20]. Therefore, this study aims to determine the Hepatitis B and / or C Infection and Clinical Staging of HIV Infected Children and the Prevalence of Immunologic Suppression.

Materials and Method

Study Area

The study was done at the Irrua Specialist Teaching Hospital (ISTH), Irrua, 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. Esan Central LGA has an estimated population of 128,571 and covers a land area of 436 square kilometers [21]. 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 has 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, $z = \alpha$ normal standard deviation for the required level of confidence usually set at 1.96, and d = tolerable margin of error usually set at 5% [22]. Substituting the 15.0% combined prevalence of HBV and HCV co-infection in HIV infected adults reported from a case-control study in Lagos [16].

$$n = \frac{0.150 \times 0.850 \times 1.962 = 195.9}{0.05^2}$$

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 Nf = n/1 + (n/N) and substituting [23].

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, In this method, socioeconomic status is determined by finding the average of the educational attainment and occupation of the mother and the father [24]. 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 red colour in both the controls and patient's windows indicated a positive result while presence of the red colour only in the control's window indicated a negative result. 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. Two documented HIV DNA PCR positive tests done earlier at the IHVN laboratory for those aged 6-18 months confirmed HIV infection.

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). This is a rapid chromatographic immunoassay for the qualitative detection of Hepatitis B surface antigen. It has a relative sensitivity greater than 99%. The test utilizes a combination of monoclonal and polyclonal antibodies to selectively detect elevated levels of HBsAg in whole blood, plasma or serum. The tests and results' interpretation were carried out according to the manufacturers' instructions while observing universal precautions. Reactive tests were repeated at least once to avoid false positive results.

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). This test uses recombinant proteins and synthetic peptides derived from core and structural regions of HCV to detect the presence of anti-HCV in plasma samples. The cost of assay for HCV infection was borne by the Researcher as this is not subsidized for children by the IHVN laboratory at ISTH. The test line region of the strip is pre-coated with recombinant HCV antigen and the test is based on chromatographic capillary migration to form a colour line. The presence of the colour line after 10 minutes indicated a positive result and its absence a negative result. The test was carried out and interpreted according to the manufacturer's instructions. Reactive tests were repeated at least once to avoid false positive results.

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 [19, 25].

Data Analysis

Data was analyzed with International Business Machines Corporation Statistical Product and Service Solution (IBM SPSS) Version 20.0 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 [26]. 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

Socio-Demographic 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. Subjects 38 (44.2%) 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 ($p = 0.440$). Subjects 34 (39.5%) 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 I). 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 ($p = 0.565$).

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

Variable	Status	Subjects	Controls	$\chi 2$	*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.

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 II. 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.

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

Clinical feature	Subjects (%)	Controls (%)	$\chi 2$	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.

The prevalence of risk factors for infection with HBV or HCV in the Subjects and Controls is shown in Table III. 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 3: Prevalence of Risk Factors for Infection with HBV or HCV in Subjects and Controls

Risk Factors	Subjects (N =86)	Controls (N=86)	$\chi 2$	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.

Clinical Stage of HIV Infection and the Prevalence of Immunologic Suppression in the Subjects

This is shown in Table IV, for the two groups of Subjects (≤5 years old versus older Subjects) for ease of comparison. 38 (28%) Subjects were ≤5 years old. 5 (13.2%) were in clinical stage 1, 6 (15.8%) in stage 2, 17 (44.7%) in stage 3 and 10 (26.3%) in stage 4. 21 (55.3%) had no immune suppression while 7 (18.4%) had moderate and 10 (26.3%) severe immune-suppression. 48 Subjects were more than 5 years of age. 1 (2.1%) was in clinical stage 1, 15 (31.3%) in stage 2, 19 (39.6%) in stage 3 and 13 (27.1%) in stage 4. 25 (52.1%) had no immune suppression while 19 (39.6%) had moderate and 4 (8.3%) severe immune suppression. 27 (71.1%) of those under 5 years old versus 32 (66.7%) older Subjects were in clinical stages 3 and 4. 17 (44.7%) versus 23 (47.9%) had moderate – severe immune suppression while among those with immune suppression, 10/17 (58.8%) versus 4/23 (17.4%) had severe immune suppression. There was no statistically significant difference between under 5s and older Subjects in the number in stages 3 and 4 illness ($\chi 2 = 0.19$, $p = 0.663$) or in the number with immune suppression ($\chi 2 = 0.09$, $p = 0.970$). However, the prevalence of severe immune suppression was significantly higher among the under-5 Subjects with immune suppression ($\chi 2 = 7.38$, $p = 0.007$; OR (95% CI) = 6.79 (1.60, 18.86)). The two subpopulations of Subjects were combined in order to meet the requirements for statistical analysis of the relationship between the clinical stage of illness and the degree of immune suppression. 12 (44.4%) of stages 1 and 2 versus

28 (47.5%) of stages 3 and 4 Subjects had immune suppression while among those with immune suppression, 4 (33.3%) versus 10 (35.7%) had severe suppression. The differences were not statistically significant ($\chi 2 = 0.07$, $p = 0.795$ for the difference in the number with immune suppression and Fisher exact $p>0.999$ for the difference in the number with severe suppression).

Table 4: Clinical Stage and the Prevalence and Severity of Immune Suppression in Subjects

Clinical stage of infection									
	None		Moderate		Severe		Total		Total (%)
	≤5	>5	≤5	>5	≤5	>5	≤5	>5	
Stage 1	3	1	1	0	1	0	5	1	6 (7.0)
Stage 2	2	9	2	5	2	1	6	15	21 (24.4)
Stage 3	12	7	2	10	3	2	17	19	36 (41.9)
Stage 4	4	8	2	4	4	1	10	13	23 (26.7)
Total	21	25	7	19	10	4	38	48	86 (100.0)
Grand total (%)	46 (53.5)		26 (30.2)		14 (16.3)		86 (100.0)		

Note: the assumptions for a group $\chi 2$ test were not met by the numbers in this Table.

Note: Percentages are in brackets, and add downwards for total and across for the grand total.

Prevalence of HBV and HCV Infections in Subjects and Controls

Table V shows the prevalence of HBV and HCV infections among the Subjects and Controls. The prevalence is also illustrated in Figure 1. 9 (10.5%) Subjects and 5 (5.8%) Controls had infection with HBV while 4 (4.7%) versus 3 (3.5%) had infection with HCV. Overall, 13 (15.1%) of the Subjects and 8 (9.3%) of the Controls had infection with either HBV or HCV. None of the Subjects or Controls had infection with both HBV and HCV. There was no statistically significant difference between the Subjects and Controls in the prevalence of HBV or HCV infection (OR (95% CI) of HBV in the Subjects = 1.89 (0.61, 5.90), $\chi 2 = 1.24$, $p = 0.266$; OR (95% CI) of infection with HCV = 1.35 (0.21, 6.22), $\chi 2 = 0.15$, $p = 0.700$). There was also no significant difference between the Subjects and Controls in the overall prevalence of infection with HBV or HCV (OR (95% CI) = 1.74 (0.68, 4.43), $\chi 2 = 1.34$, $p = 0.245$). The OR (95% CI) of the ratio of HBV to HCV infection in the Subjects versus Controls (2.25:1 versus 1.67: 1) was 1.35 (0.21, 8.62), $\chi 2 = 0.10$, $p = 0.751$. All subsequent analyses were done with the prevalence of HBV combined with that of HCV, because no Subject or Control had both infections neither was there a significant difference between the Subjects and Controls in the prevalence of infection with either virus.

Table 5: Prevalence of Infection with HBV and HCV in Subjects and Controls

Status	N	No (%) positive for		Total (%) positive
		HBV	HCV	(HBV + HCV)
Subjects	86	9 (10.5)	4 (4.7)	13 (15.1)
Controls	86	5 (5.8)	3 (3.5)	8 (9.3)
$\chi 2$/p		1.24/0.266	0.15/0.700	0.10/0.245

Note: Percentages are in brackets and add across.

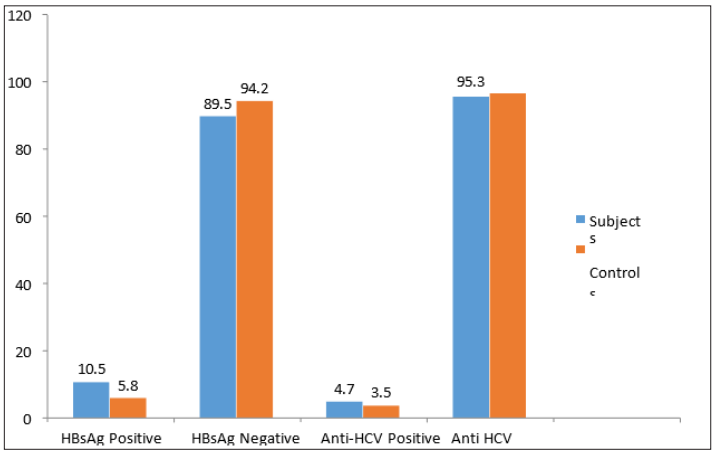

Figure 1: Prevalence of HBV and HCV Infections in Subjects and Controls

Relationship Between the Prevalence of HBV and HCV Infections and Gender, Age, Socioeconomic Status and Ethnic Group

This is shown in Table VI for age and gender and in Table VII for socioeconomic status and ethnic group. The relationship with gender is also illustrated in Figure 1. 4 (8.9%) male Subjects and 2 (4.0%) male Controls and 5 (12.2%) female Subjects and 3 (8.3%) female Controls had HBV infection. The corresponding figures for HCV infection were 3 (6.7%) versus 2 (4.0%) in males and 1 (2.4%) versus 1 (2.8%) in females. There was no statistically significant difference between the Subjects and Controls in the prevalence of HBV or HCV infection in males ($\chi 2 = 1.32$, $p = 0.251$) and females (Fisher exact $p = 0.910$) (Table VI and Figure 1). There was no significant difference between the prevalence of HBV and HCV in under-5 Subjects and Controls (7.8% versus 4.2%, Fisher exact $p = 0.779$) or in older Subjects and Controls (20.8% versus 15.8%, $p = 0.551$) (Table VI). The difference in the prevalence of HBV and HCV between the Subjects and Controls from the lower socioeconomic group approached statistical significance (17.3% versus 7.0%, $p = 0.077$) while the difference between non-Esan Subjects and Controls was significant (0.0% versus 20.7%, $p = 0.014$) (Table VII). None of the other differences between Subjects and Controls were statistically significant.

Table 6: Age and Gender and the Prevalence of Infection with HBV and HCV in Subjects and Controls

Status	N	No. (%) infected with HBV or HCV	OR (95% CI) of the prevalence infection in the Subjects	$\chi 2$/p
Male Subjects	45	7 (15.6)	2.12 (0.58, 7.78)	1.32/0.251
Male Controls	50	4 (8.0)		
Female Subjects	41	6 (14.6)	1.37 (0.76, 5.31)	0.910*
Female Controls	36	4 (11.1)		
Subjects ≤5 years old	38	3 (7.8)	1.97 ((0.31, 12.44)	0.779*
Controls ≤5 years old	48	2 (4.2)		
Subjects >5 years old	48	10 (20.8)	1.40 (0.46, 4.28)	0.36/0.551
Controls >5 years old	38	6 (15.8)		

Note: Percentages are in brackets and read across. OR (95% CI) = odds ratio (95% confidence interval). *Fisher exact test.

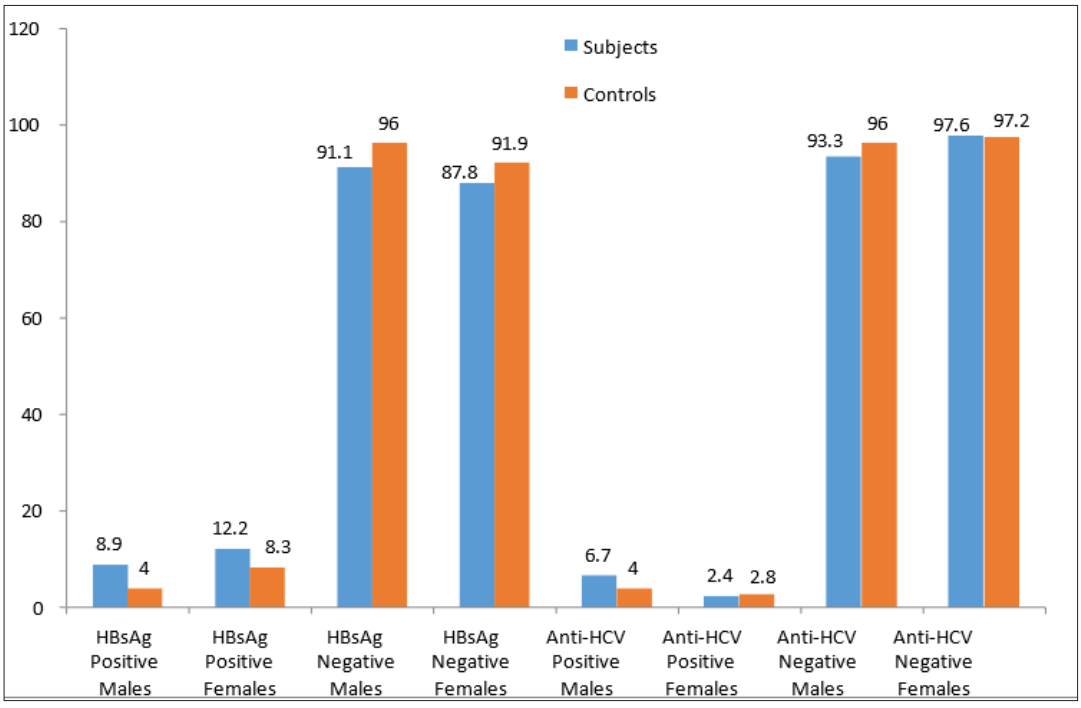

Figure 2: Prevalence of HBV and HCV infections in Subjects and Controls by Gender

Table 7: Socioeconomic Status and Ethnic Group and the Prevalence of Infection with HBV and HCV in Subjects and Controls

Status	N	No. (%) infected with HBV or HCV	OR (95% CI) of the prevalence of infection in the Subjects	χ2/p
USEC Subjects	34	4 (11.8)	0.53 (0.10, 2.75)	0.725*
USEC Controls	15	3 (20.0)		
LSEC Subjects	52	9 (17.3)	2.76 (0.87, 8.80)	3.14/0.077
LSEC Controls	71	5 (7.0)		
Esan Subjects	52	9 (17.3)	1.78 (0.59, 5.40)	1.05/0.307
Esan Controls	57	6 (10.5)		
Non-Esan Subjects	34	0	NA	0.014*
Non-Esan Controls	29	6 (20.7)		

Note: Percentages are in brackets and read across. OR (95% CI) = odds ratio (95% confidence interval). *Fisher exact test.

NA = not applicable. USEC = Upper Socioeconomic Classes, LSEAC = Lower Socioeconomic Classes.

Table 8: Mean ± SD of CD4+ Count of HBV or HCV Infected and Non-Infected Subjects and Controls

Status	Subjects		Controls		t/p
	N count	Mean ± SD CD4+	N count	Mean ±SDCD4+ Count	
HBV/HCV +ve	10	908.1 ± 510.2	6	1087.7 ± 571.9	0.65/0.525
HBV/HCV -ve	38	1087.6 ± 513.4	32	1287.2 ± 612.9	1.48/0.143
t/p	**0.98/0.330**		**0.74/0.465**		

Table 9: Mean ± SD of CD4 Percent of HBV or HCV Infected and Non-Infected Subjects and Controls

Status	Subjects		Controls		t/p
	N percent	Mean ± SD CD4	N percent	Mean ± SD CD4	
HBV/HCV +ve	3	25.0 ± 19.3	2	37.8 ± 23.7	0.67/0.550
HBV/HCV -ve	35	26.5 ± 13.2	46	46.4 ± 72.1	1.61/0.111
t/p	0.18/0.859		0.17/0.868		

HBV = hepatitis B virus, HCV = hepatitis C virus, +ve = positive, -ve = negative.

Discussion

The prevalence of infection in this study was higher in female children with HIV infection (12.2% versus 8.9% in males). The trend was similar in the controls (8.3% versus 4.0%). The reason for this difference is not clear. The observation is at variance with previous reports in adult populations from Jos, North Central Nigeria and India [11,13,27]. The authors of these reports suggested that males were more likely to be HIV/HBV positive because they have multiple sexual partners and also practice unprotected sex in largely polygamous settings [13]. The present study was in children and it will be inappropriate to draw similar comparisons with the results from studies in adults as the sexual orientation of the children in this setting was not assessed.

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, the 4.9% reported from Chinese children and the 7.7% reported recently from nearby Benin City, Nigeria [28-30]. It is, however, lower than the 12.1% reported from Cote d'Ivoire [31]. 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 [15]. 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 [31].

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, Audu et al., reported a 0.0% prevalence in preschool children, while another study in Benin City, Nigeria reported a prevalence of 0.25% [30,37]. 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% [13]. On the other hand, no co-infection with HCV was reported from Ilorin [11].

The prevalence of HCV in HIV-infected children in previous reports from developing countries ranges from a very low prevalence of 0.0% in Cote d'Ivoire through 5.2% in Benin City, Nigeria and 9.6% in China to a high prevalence of 13.8% in Tanzania [31,38-40]. The 4.7% prevalence in HIV-infected children in this study is within this range and comparable to the prevalence reported recently from Benin City [38]. It is higher than the prevalence of 2.3% reported from Abuja, Nigeria but also comparable to the 4.8% reported from HIV-infected adults in Ibadan, Nigeria [16,30]. Other studies from Northern Nigeria have reported prevalence rates of 0.0%-8.2% in HIV-infected adults [13,30]. As with the prevalence of HBV in HIV-infected patients, it is clear that the prevalence of HCV infection also varies between locales even within the same sub-region and the same country.

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 as discussed earlier reported an HCV prevalence of 0.25% in the general population of children [30,38]. 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, including Nigeria [28,30,31]. It is also in keeping with the low prevalence reported from HIV-infected Zambian adults and from blood donors in Ilorin, Nigeria [12]. 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 and 7.25% from Jos [13,16]. 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 [41,42].

Conclusion

This study emphasizes the prevalence of hepatitis B and/or C co-infection in HIV-infected children and its correlation with clinical staging and immunological suppression. The results show that compared to children without hepatitis co-infection, co-infected children were more likely to present with advanced clinical stages of HIV and a higher prevalence of immunologic suppression. This emphasizes how viral hepatitis has a major effect on the course and clinical results of HIV in children. The high reported prevalence of immunologic suppression highlights this population's sensitivity and the necessity of comprehensive management, early discovery, and ongoing monitoring. To enhance long-term health outcomes, routine hepatitis B and C screening in children with HIV should be given priority, coupled with early antiretroviral medication initiation and hepatitis-specific therapies. Reducing the morbidity and mortality linked to HIV-hepatitis co-infection in children requires bolstering public health initiatives such as immunization, preventive education, and improved access to diagnostic services.

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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