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Variation of CD4$^+$ T-Lymphocyte Counts and Transaminases in HIV and HIV/HBV Co-infected Patients on Therapy at Nylon Hospital Douala, Cameroon

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Authors' contributions

We declare that this work was carried out by us and all liabilities pertaining to claims relating to the content of this article will be borne by us. Author JCNA participated in the design, analysed the data, drafted the manuscript and substantially revised it, author SDM participated in data collection, contributed in the Write-up and substantially revised the manuscript. Authors DSN JNP and ECM participated in the design, Write-up and substantially revised the manuscript for academic content.

Original Research Article

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ABSTRACT

Objective: This study was aimed at determining the prevalence of Hepatitis B and associated risk factors such as CD4$^+$ counts variation and liver enzymes among HIV co–infected patients and those with HIV mono-infections only.
Design and Methods: Three hundred and fourteen (314) HIV patients took part in this

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cross sectional case control study. Socio-demographic information and history of exposure to risk factors such as scarification, blood transfusion, and unprotected sexual intercourse and alcohol consumption, were obtained through a well-structured questionnaire. Serological tests were done to determine the presence of Hepatitis B (HB) surface Antigen, liver enzymes' activities were estimated and $CD4^+$ cell counts evaluated using standard laboratory methods.

Results: Out of the 314 HIV patients, 20 (6.4%) tested positive for hepatitis B surface antigen (HBsAg) while 294 (93.6%) were negative. Most HIV patients co–infected with HBV were in the age group 31 to 45 years. There was no significant variation when co-infection and mono-infection groups were compared based on age and sex (p=0.7405 and p=0.3361). More males, 7 (2.23%) against 2 (0.64%) females (P=0.02) co–infected with HBsAg had a $CD4^+$ cell counts in the range 201-350cells/µL. No significant difference of liver transaminases (SGPT and SGOT) levels between mono and co-infection groups (P>0.05) was observed. No association of HBsAg with observed risk factors among HIV patients was noted.

Conclusion: The study concluded that the prevalence of hepatitis B among HIV patients was 6.4% with majority of the patients having $CD4^+$ cell counts within 201-350. The liver function parameters (transaminases) were not affected with HIV/HBV co-infection.

Keywords: HBV; HIV; $CD4^+$ cell counts; SGPT; SGOT.

1. INTRODUCTION

In today's setting of highly active anti-retroviral therapy in Human Immuno Deficiency Virus (HIV) infected patients, there is increasing incidence of liver injury, life threatening hepatotoxicity events and end stage liver disease [1]. This could be due to the wide use of anti-retroviral therapy [2] or as a result of co-infection with liver diseases [3,4]. This brings about the need to evaluate hepatitis B (HB) and HIV co-infection in order to determine the cause of liver disease and maximize treatment outcome.

Sub Saharan Africa accounts for the highest burden of HIV infection worldwide with hepatitis B contributing significantly to morbidity [2]. HIV and Hepatitis B share risk factors for viral acquisition; hepatitis B is more common in HIV than in the general population [5,6]. In North America, Australia and Europe with low endemicity of both HB and HIV, prevalence rates of chronic co-infection are between 5-7% among HIV-infected individuals [7]. The predominant transmission routes are percutaneously and through sexual intercourse and infection occurs mostly in adults [8]. While in countries with intermediate and high endemicity, HIV/HB co-infection rates are as high as 10 to 20% with a predominance of perinatal transmission and infection around childhood [9-11].

Studies carried out in parts of sub Saharan Africa revealed significant co-infection rates. In a study in Kenya, 23 out of 378 persons living with HIV were tested positive for hepatitis B making up for a co-infection prevalence of 6.1% [12]. In Gambia, the HBsAg prevalence detected in HIV infected individuals was 12.2% [13]. In the North West region of Cameroon, HIV infected patients showed a prevalence of 12.6% of co-infection with HBV [14].

According to personal observations, most patients attending local hospitals do not test for hepatitis B before beginning HAART for HIV. During the course of the disease, increases in liver enzyme level is hardly attributed to a probable HBV infection especially when symptoms are not evident, thus patients treatment is change without consideration for possible co-infection. This may result to increase in liver disease progression. As HAARTs become

current in parts of Africa with high endemic city of HBV infection, such as Cameroon, long term survival increases in HIV infected patients, giving way to the development of chronic HB in HIV infected patients than before [3]. Thus in order to be able to monitor liver diseases in resource limited countries, there is need to evaluate HB/HIV co-infection. Thus assessing hepatitis B and HIV co-infection is primordial as a basis for decisions concerning treatment regimens.

2. MATERIALS AND METHODS

This was across sectional study which lasted three months from September to December, 2012. Consecutive HIV/AIDS patients (as determined by positive results of enzyme-linked immune sorbent assay (ELISA) and Western blot assay) visiting for routine $CD4^+$ T-Lymphocytes counts and liver enzymes function test were enrolled in the study from the HIV/AIDS Treatment Centers at the Nylon District Hospital Douala situated in the Littoral region of Cameroon. Their informed consent was obtained and an ethical clearance and authorization to collect specimens and data for research was obtained from the Regional Delegation of Public Health for the Littoral region of Cameroon. An estimate of the required number of participants was obtained using a formula for estimating sample size for proportions. The prevalence used in this formula was derived from a previous work [14]. Participants were of both sex ageing between 02 and 66 years. Children less than 02 years of age were excluded. Information on demographic data was obtained from patients or guardians (for those less than 18y.o.) through a well-structured questionnaire. About 4ml of fasting blood was collected by venipuncture into an EDTA and a dry tube per patient. The EDTA tubes were allowed to stand for about 20 minutes then homogenized gently before analyzing for $CD4^+$ cells using Partec Cyflow® counter following manufacturers' procedure. The dry tubes were centrifuged at 3000rpm for 5 minutes to obtain serum for Serum Glutamate Pyruvate transaminase (SGPT), Serum Glutamate Oxalate transaminase (SGOT) analysis according to the 2002 International Federation of Biochemistry protocol [15] using the commercial kit manufacture by CYPRESS DIANOSTICS Ltd. Polyclonal anti-HBsAg antibodies on a test strip were used to determine HBsAg using standard protocols. Data were entered into Microsoft excel through which was used for sorting into different groups and analysed using the statistical package Statxact Version 3.2. Kolmogorov-Smirnov test was done to check for normality. Participants were grouped as co-infected and mono-infected. Differences between groups of $CD4^+$ counts, SGOT and SGPT values were compared using Fisher exact test. While the Spearman's correlation test was used to establish association between risk factors. Statistical significance was set at $p \leq 0.05$.

3. RESULTS

In this study, out of the 314 HIV positive patients, 88 were males while 226 were females giving a male/ female ratio of 1:2.6. Of these, there were 20 (6.37%) co-infected and 294 (93.63%) mono-infected patients. Participants in the study were grouped into four different age groups, ≤15 years, 7(2.23%); 16-30 years, 40(12.74%); 31-45 years, 178(56.69%) and ≥46 years, 89 (28.34%) as shown in Table 1. below. The co-infection prevalence among the different age groups were 0.0%, 1.27%, 4.14% and 0.96% respectively see Table 2. There was no significant variation in co-infection and mono-infection prevalence by age and among males participants in the study population (χ^2=1.250, p=0.7405, df=3) and among females (χ^2=3.243, p=0.3361, df=3) as shown in Table 2 below.

$CD4^+$ T lymphocytes counts in the study population were grouped into four classes (≤200, 200 to 350, 351 to 500 and >500) and variation observed in co-infection and mono-infection. In the entire population, 47(14.9%) mono-infected had $CD4^+$ counts ≤200cells/mm^3

compared to 1(0.32%) co-infected. Considering $CD4^+$ cell counts within 201-350, the prevalence of co-infections was significantly higher in male than in female HIV patients (p=0.02) as shown in Table 3 below.

Table 1. Socio-demographic characteristics of study subjects

Socio-demographic characteristics of study subjects			
	Mono-infectionn=294(93.63%)	**Co-infection n=20 (6.37%)**	**Total**
Sex			
Female	215(73.1)	11(55)	226
Male	79(26.9)	9(45)	88
Age groups			
<15	7(2.4)	0(0)	7
16-30	36(12.2)	4(20.0)	40
31-45	165(56.1)	13(65.0)	178
46>	86(29.3)	3(15.0)	89
Marital status			
Married	156(53.1)	9(45.0)	165
Single	103(35.0)	9(45.0)	112
Widow/widower	35(11.9)	2(10.0)	37
Alcohol			
Yes	181(61.6)	11(55.0)	192
Risk factors			
Unprotected sex	128(43.5)	9(45.0)	137
Scarifications	70(23.8)	4(20.0)	74
Blood transfusion	30(10.2)	0(0)	30

Table 2. Prevalence of co-infection by age and by sex

Sex	**Age classes**	**Prevalence by age and sex**								
		Co-infection		**Mono-infection**		**Total**		**Statistical comparisons**		
		n	**%**	**n**	**%**	**n**	**%**	**χ^2**	**df**	**p-value**
A, Males	<15	0	0.00	4	1.27	4	1.27	1.250	3	0.7405
	16-30	1	0.32	8	2.55	9	2.87			
	31-45	6	1.91	38	12.10	44	14.01			
	46>	2	0.64	29	9.24	31	9.87			
	Total	9	2,87	79	25.16	88	28.03			
B, Females	<15	0	0.00	3	0.96	3	0.96	3.243	3	0.3361
	16-30	3	0.96	28	8.91	31	9.87			
	31-45	7	2.23	127	40.44	134	42.67			
	46>	1	0.32	57	18.14	58	18.47			
	Total	11	3.50	215	68.47	226	71.97			
Pooled data	<15	0	0.00	7	2.23	7	2.23	2.607	3	0.4023
	16-30	4	1.27	36	11.46	40	12.74			
	31-45	13	4.14	165	52.55	178	56.69			
	46>	3	0.96	86	27.39	89	28.34			
	Total	20	6.37	294	93.63	314	100			

Statistical comparisons A vs. B: fisher freeman–Halton exact Test Co-infection: X^2=1.260, p=0.6782, df=3 /Mono-infection: X^2=6.701, p=0.0709, df=3

Based on SGPT and SGOT, there was no significant difference in high and normal values in co-infection and mono-infection between males and females (p>0.05) as shown in Tables 4 and 5 below. It was observed that in the entire study population, there was no significant association between risk factors (blood transfusion, unprotected sex, scarification and alcohol consumption) and HIV/Hepatitis B co-infections as shown in Table 6 below.

Table 3. Variation of $CD4^+$ T lymphocyte counts

$CD4^+$ count	Female				Male				Total				Statistical comparisons		
	Mono-infection		Co-infection		Mono-infection		Co-infection		Mono-infection		Co-infection				
	n	%	n	%	n	%	n	%	n	%	n	%	χ^2	df	p-value
≤200	24	7.64	1	0.32	23	7.32	0	0	47	14.97	1	0.32	0.8759	1	1.0000
201-350	43	13.7	2	0.64	22	7.01	7	2.23	65	20.7	9	2.87	6.069	1	0.0238
350-500	92	29.3	4	1.27	23	7.32	1	0.32	115	36.62	5	1.59	0.1707	1	1.0000
≥500	56	17.83	4	1.27	11	3.5	1	0.32	67	21.34	5	1.59	0.3422	1	1.0000
Total	215	68.47	11	3.5	79	25.16	9	2.87	294	93.63	20	6.37	3.006	1	0.1190

Co- infection and mono-infection among males: χ^2=8.257, p=0.0192, df=3; Co-infection and mono-infection among Females: χ^2=0.7331, p=0.9137, df=3; Co-infection and mono-infection: χ^2=5.885, p=0.1052, df=3; Co-infection: X^2=6.713, p=0.0705, df=3 /Mono-infection: X^2=18.78, p=0.0003, df=3

Table 4. Variation of SGPT in Co-infected and mono-infected patients

SGPT values	Female				Male				Total				Statistical comparisons		
	Mono-infection		Co-infection		Mono-infection		Co-infection		Mono-infection		Co-infection				
	n	%	n	%	n	%	n	%	n	%	n	%	χ^2	df	P-value
Normal	95	30.25	3	0.96	41	13.06	3	0.96	136	43.31	6	1.91	1.206	1	0.3739
High	120	38.22	8	2.55	38	12.1	6	1.91	158	50.32	14	4.46	2.411	1	0.1964
Total	215	68.47	11	3.51	79	25.16	9	2.87	294	93.63	20	6.37	3.006	1	0.1190

Co-infection and mono-infection among males: χ^2=1.079, p=0.4839, df=3; Co-infection and mono-infection among Females:χ^2=1.129, p=0.3570, df=3; Co-infection and mono-infection: χ^2=5.885, p=0.1052, df=3; Mono-infection: X^2=1.383, p=0.2912, df=1; Co-infection: X^2=0.1801, p=1.000, df=1

Table 5. Variation of SGOT in Co-infected and mono-infected patients

SGOT value	Female				Male				Total				Statistical comparisons		
	Mono-infection		Co-infection		Mono-infection		Co-infection		Mono-infection		Co-infection				
	n	%	n	%	n	%	n	%	n	%	n	%	χ^2	df	P-value
Normal	63	20.06	1	0.32	22	7.01	1	0.32	85	27.07	2	0.64	0.995	1	0.4611
High	152	48.41	10	3.18	57	18.15	8	2.55	209	66.56	18	5.73	2.405	1	0.1714
Total	215	68.47	11	3.5	79	25.16	9	2.87	294	93.63	20	6.37	3.006	1	0.1190

Co- infection and mono-infection among males: χ^2=1.172, p=0.4357, df=3; Co- infection and mono-infection among Females: χ^2=1.867, p=0.1869, df=3; Co-infection and mono-infection: χ^2=3.331, p= 0.0744, df=3; Mono-infection: X^2=0.05418, p=0, 0.8850, df=1; Co-infection: X^2=6.713, p=0.0705, df=1

Table 6. Assessing the association between Risk factors and HIV/HBV co-infection

Risk factors	Mono-infection		Co-infection		Total	Statistical comparisons p-value
	N(294)	%	N(20)	%	N(314)	1.000
Unprotected sex						
Yes	128	43.54	9	45	137	
No	166	56.46	11	55	177	
Scarifications						0.7931
Yes	70	23.80	4	20	74	
No	224	76.20	16	80	240	
Blood transfusion						0.2362
Yes	30	10.20	0	0	30	
No	264	89.80	20	100	284	
Alcohol consumption						0.6374
Yes	181	61.6	11	55	192	
No	113	38.4	9	45	122	

4. DISCUSSION

In this study we observed that among the 314 HIV positive participants enrolled, 20 were positive for HBsAg giving a HIV/HBV co-infection of 6.37%. This prevalence was comparable to that obtained in Kenya 6% [16]. In another study involving young adults and adolescent in Uganda, the prevalence was 6.1% [17] and in Tanzania 6.5% [18]. This similarity in results could be due to similarities in socio demographic characteristics of the different settings characterized by intermediate and high HBV endemicity where the principal routes of HBV transmission are perinatal or in early childhood; thus, HBV infection usually precedes HIV infection by decades [16].

The prevalence rate obtained was however lesser when compared with two previous Cameroonian studies where the prevalence of HIV/HBV co-infection was 12.5% [19,20]. This difference in prevalence rates between our study and the later could be as a result of differences in sampling techniques and study site but probably of the increasing awareness about HBV transmission risk factors. We equally observed that co-infection prevalence, though not statistically significant, was greater in males than in females. This is similar to results obtained in Nigeria [21] and could be explained by the fact that males are more exposed to risk factors such as multiple sex partners in polygamous homes and unprotected sexual intercourse.

The study showed that the prevalence of hepatitis B was 0.0% among HIV patients aged ≤15. This could be explained largely by the fact that only a minimal proportion of participants fell in the age group because studies carried out in pediatric HIV/HBV co-infection show relatively high rates. Such rates as 7.7% and 10.0% have been observed in studies in Nigeria [22] and in Cote d'Ivoire [23]. In this age group, transmission of co-infection is mostly perinatal or in early infancy. If a pregnant woman is an HBV carrier and is also hepatitis B e antigen (HBeAg)-positive, there is 90% chance that the baby will be infected and become a carrier. Among the infected children, about 25.0% may die at adulthood from liver disease or liver cancer [24]. We observed that the prevalence of co-infections was higher among patients within the age range of 31–45 year, followed by those within the age bracket of 16–30 years. This high prevalence rates can be attributable to diverse risk factors that come with

age such as receiving blood and/or blood products, tattoos and other skin-piercing activities, unprotected penetrative sex, in particular anal and vaginal sex and health care and occupational risks.

We found out that most of the participants in the study had $CD4^+$ T- lymphocytes counts between 351 to 500cells/mm^3 of blood with no significant difference in $CD4^+$ counts in mono-infected and co-infected males and females Table 3. This could be explained by the fact that patients generally began treatment at lower $CD4^+$ counts; once started, follow up is taken seriously, reason why most patients may not have lower $CD4^+$ counts. The highest number of co-infected patients had $CD4^+$ cell counts within 201 to 350. Similar findings were obtained in Uganda on Hepatitis B virus infection in adolescents and young adults with co-infection strongly associated with HIV clinical stage I and II [17]. A significant difference between co-infected and mono-infected males and females was noted, with more males' co-infected at lower level of $CD4^+$ counts. This is contrary to what was observed in Uganda were more females were co-infected [17], probably due to the difference in sample size selection criteria. In mono-infection, females were found to have higher counts than males differing with a the study carried out in Kenya where no significant difference in $CD4^+$ counts between patients with HIV alone and co-infected HIV and HBV [16] was observed [6].

We found out from this study that there was an elevation of liver enzymes among all the patient groups, though there was no significant association in co-infected and mono-infected participants. Most participants had raised SGPT and SGOT levels with no significant difference in co-infected and mono-infected nor among males and females Tables 4 and 5. These findings were similar with those from two other studies performed in Nigeria [25] and in Kenya [16].

From the questionnaire analyses, we observed that the most frequently observed risk factors were unprotected sexual intercourse, blood transfusion, alcohol consumption and scarification. However when closely analysed we saw that there was no significant association between the observed risk factors and co-infection Table 6respectively. These findings are similar with those from a study in Nigeria which equally found no association between commonly known risk factors and co-infection [22]. The lack of association between some of these practices and transmission of HBsAg can be attributed to the current drive for awareness of HIV, which has increased knowledge of prevention methods.

5. CONCLUSION

This study showed that there was an important prevalence of hepatitis B in HIV infected patients and that there was no significant difference in distribution of co-infection by age and by sex. It equally showed that patients' $CD4^+$ T-lymphocyte count was not affected by mono-infection and co-infection. Mono-infection and co-infection alike did not affect liver function parameters (SGPT and SGOT).

6. RECOMMENDATION

There is thus a need for proper screening of HIV/HBV co-infection before placing patients on HAART and even during follow up of patients, to limit drug resistance and liver disease progression.

Governments and local bodies in the elaboration of HIV prevention programs should include subvention of hepatitis B screening so as to facilitate early diagnosis of hepatitis B in HIV patients in order to initiate treatment early.

CONSENT

Informed consent was sought and gained from each HIV/AIDS patient visiting the HIV/AIDS Treatment Centers at the Nylon District Hospital Douala, Cameroon before his/her involvement in the study.

ETHICAL APPROVAL

Ethical clearance was obtained from the Regional Delegation of Public Health of the Littoral Region in Douala, Cameroon.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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