

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

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 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 ~~in HB~~ 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 201-350 cells/μL. ~~There was~~ No significant difference ~~when of~~ liver transaminases (SGPT and SGOT) levels ~~were compared~~ between mono and co-infection groups (P > 0.05) was observed. ~~There was~~ 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. ~~No association of~~ The liver function parameters (transaminases) were not affected with HIV/HBV co - infection ~~was observed~~.

27 **KEYWORDS:** HBV; HIV; CD4⁺ cell counts; SGPT; SGOT

28

29 **INTRODUCTION**

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

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

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

50 According to personal observations, most patients attending local hospitals do not test for
51 hepatitis B before beginning HAART for HIV. During the course of the disease, increases in
52 liver enzyme level is hardly attributed to a probable HBV infection especially when symptoms
53 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 endemicity 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.

MATERIALS AND METHODS

This was a cross sectional study which lasted three months from September to December, 2012. Consecutive HIV/AIDS patients (as determined by positive results of enzyme-linked immunosorbent 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 18 y.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 3000 rpm 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 $p \leq 0.05$.

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 ($\chi^2 = 1.250$, $p = 0.7405$, $df = 3$) and among females ($\chi^2 = 3.243$, $p = 0.3361$, $df = 3$) 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 ≤ 200 cells/mm³ 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.

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 table 4 and 5 below. It was observed that in the entire study population, there was no significant

108 association between risk factors (blood transfusion, unprotected sex, scarification and alcohol
109 consumption) and HIV/Hepatitis B co-infections as shown in Table 6 below.

110 DISCUSSION

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

119 ~~The Pp~~prevalence rates ~~from the study~~obtained ~~were~~was however lesser when compared with
120 two previous Cameroonian studies where the prevalence of HIV/HBV co-infection was 12.5
121 % [19, 20]. This difference in prevalence rates between our study and the later could be as a
122 result of differences in sampling techniques and study site but probably of the increasing
123 awareness about HBV transmission risk factors. We equally observed that co-infection
124 prevalence, though not statistically significant, was greater in males than in females. This is
125 similar to results obtained in Nigeria [21] and could be explained by the fact that males are
126 more exposed to risk factors such as multiple sex partners in polygamous homes and
127 unprotected sexual intercourse.

128 The study showed that the prevalence of hepatitis B was 0.0 % among HIV patients aged ≤ 15 .
129 This could be explained largely by the fact that only a minimal proportion of participants fell
130 in the age group because studies carried out in pediatric HIV/HBV co-infection show
131 relatively high rates. Such rates as 7.7% and 10.0% have been observed in studies in Nigeria
132 [22] and in Cote d'Ivoire [23]. In this age group, transmission of co-infection is mostly

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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, 13 (4.14%) followed by those within the age bracket of 16 – 30 years, 4 (1.27%). 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 500 cells/mm³ of blood. However in with this class no significant difference in CD4⁺ counts ~~was observed~~ in mono-infected and co-infected males and females ($\chi^2 = 0.1707$, p -value = 1.0000 (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 a CD4⁺ cell counts within 201 to 350; this finding similar to findings were as obtained similar to that of a study in Uganda on Hepatitis B virus infection in adolescents and young adults with HIV infection in an urban clinic in a resource limited setting where co-infection was found to be strongly associated with HIV clinical stage I and II [17]. Our study showed a significant difference between co-infected and mono-infected males and females ($\chi^2 = 6.069$, p -value = 0.0238 was noted). W with more males' co-infected at lower level of CD4⁺ counts. This is contrary to what was observed in Uganda where more females were co-infected [17], probably due to the difference in sample size selection criteria. CD4⁺ counts distribution varied differently in co infected and mono infected males with higher values in mono infection than in co infection ($\chi^2 = 8.257$, $p = 0.0192$). In mono-infection, females were found to have higher counts than males ($\chi^2 = 18.78$, $p = 0.0003$). These findings differing ed with a

previous the study carried out in Kenya which showed where no significant difference in CD4⁺ counts between patients with HIV alone and those that had both co-infected HIV and HBV [16] co-infected patients was observed especially those with low CD4⁺ counts, are at increased risk for liver related mortality [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 ($\chi^2 = 5.885$, $p = 0.1052$, $df = 3$) nor among males and females (Tables 4 and 5). These findings were similar with those from two other studies performed, one in Nigeria [25] and another one in Kenya [16].

From the questionnaires 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 ($p = 1.000$, 0.2362 and 1.000 Table 6) respectively. 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.

CONCLUSION

This study showed that there was a high 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 counts did not vary differently based was not affected by on mono-infection and co-infection. However more co-infected males than females had CD4⁺ T lymphocyte counts between 201 and 350. Mono-infection and co-infection alike did not affect liver function parameters (Variation in SGPT and SGOT) values did not differ significantly in mono infection and co infection.

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187 **RECOMMENDATIONS**

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

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

194 **CONFLICT OF INTEREST**

195 The authors have no conflicting interest to declare.

196

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204 **Table 6:** Assessing the association between Risk factor

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276 Tables FOR THE ARTICLE

277 Table 1: Socio-demographic characteristics of study subjects

Socio-demographic characteristics of study subjects

	Mono- infection n =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

278

279 **TABLE 2: Prevalence of co-infection by age and by sex**

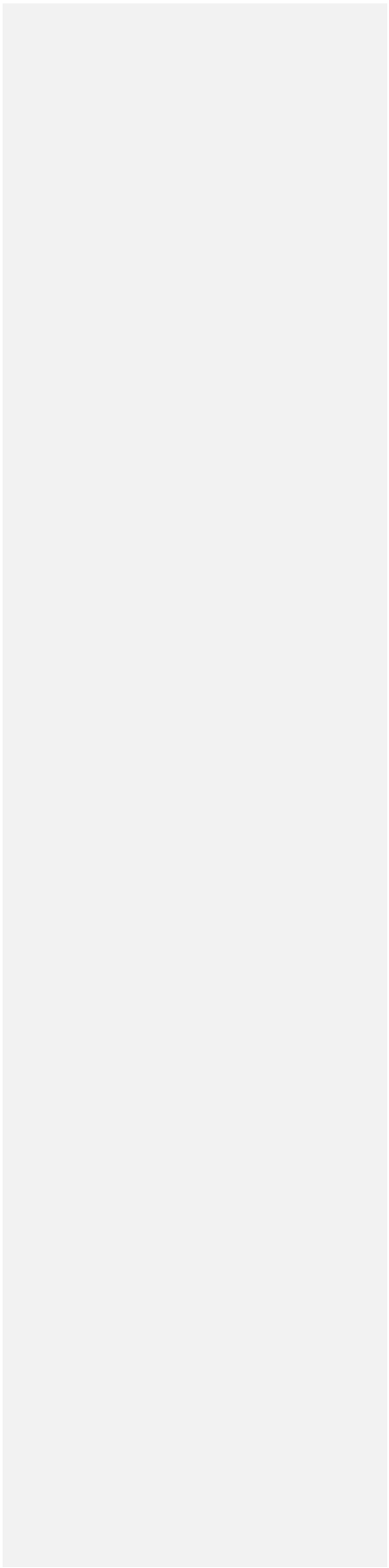
Sex	Age classes	Prevalence by Age and Sex											
		Co-infection		Mono-infection		Total		Statistical comparisons					
		n	%		n	%		n	%		χ^2	df	p-value
A, Males	<15	0	0.00		4	1.27		4	1.27		1.250	3	0.7405
	16-30	1	0.32		8	2.55		9	2.87				
	31-45	6	1.91		38	12.10		44	14.01				
	46>	2	0.64		29	9.24		31	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				

280 Statistical comparisons A vs. B: fisher freeman –Halton exact Test

281 Co-infection: $X^2= 1.260$, $p=0.6782$, $df =3$ /Mono-infection: $X^2= 6.701$, $p=0.0709$, $df =3$

282

283



285 **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		χ^2	df	p- value
	n	%	n	%	n	%	n	%	n	%	n	%			
≤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

286 Co- infection and mono-infection among males: $\chi^2= 8.257$, p= 0.0192, df = 3
287 Co- infection and mono-infection among Females: $\chi^2=0.7331$, p= 0.9137, df = 3
288 Co- infection and mono-infection: $\chi^2=5.885$, p= 0.1052, df = 3
289 Co-infection: X²= 6.713, p=0.0705, df =3 /Mono-infection: X²= 18.78, p=0. 0003, df =3
290
291

292

293

294 **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		χ^2	df	P-value
	n	%	n	%	n	%	n	%	n	%	n	%			
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

295 Co- infection and mono-infection among males: $\chi^2=1.079$, p= 0.4839, df = 3
296 Co- infection and mono-infection among Females: $\chi^2=1.129$, p= 0.3570, df = 3
297 Co- infection and mono-infection: $\chi^2=5.885$, p= 0.1052, df = 3
298 Mono-infection: $X^2= 1.383$, p=0.2912, df =1
299 Co-infection: $X^2= 0.1801$, p=1.000, df =1
300

301

302 **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

303 Co- infection and mono-infection among males: $\chi^2=1.172$, $p=0.4357$, $df=3$
304 Co- infection and mono-infection among Females: $\chi^2=1.867$, $p=0.1869$, $df=3$
305 Co- infection and mono-infection: $\chi^2=3.331$, $p=0.0744$, $df=3$
306 Mono-infection: $X^2=0.05418$, $p=0.08850$, $df=1$
307 Co-infection: $X^2=6.713$, $p=0.0705$, $df=1$
308

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

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

