

Prevalence of Hepatitis B and Tuberculosis among People Living with HIV (PLHIV) in Cameroon

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ABSTRACT

Introduction: Hepatitis B and Tuberculosis are leading causes of morbidity and mortality among people living with HIV (Human Immunodeficiency Virus). These infections are on the rise worldwide, particularly in sub-Saharan Africa. The objective of this study was to identify the presence of Hepatitis B virus and Tuberculosis among people living with HIV at the Central Hospital and Jamot Hospital in Yaoundé.

Materials and Methods: This was a cross-sectional hospital-based descriptive study in which blood and sputum samples were collected from randomly selected 362 people living with HIV (September 2017 to March 2018). Bacteriological examination with auramine staining for the detection of Acido Alcohol Resistant Bacilli, was done. This was followed by amplification of the Mycobacterium tuberculosis sequence IS6110 by a Polymerase Chain Reaction and the strips interpreted with the aid of electrophoresis. Serological analysis for the determination of Hepatitis B virus surface antigen was performed with the aid of a rapid diagnostic test. Positive cases were further confirm using an enzyme-linked immunosorbent assay. Data analysis was purely descriptive calculating prevalence and 95% confidence interval using SPSS software version 22.1.

Results: After analysis, we obtained a prevalence of 9.1% for HIV/Tuberculosis co-infection, in which men were predominant with the prevalence of 5.2% against 0.6% in women. A seroprevalence of 13.5% for HIV/Hepatitis B co-infection was obtained, with the female sex being predominant having a prevalence of 7.7% against 5.8% in males.

Conclusion: These results demonstrate the current epidemiological situation of HIV/Hepatitis B and HIV/Tuberculosis co-infections in Cameroon. Thus, these co-infection remain a real public health problem due to the increase in co-morbidity among HIV patients, hence the need for careful and constant monitoring of these infections so as to ensure better management.

Keywords: Co-infections, HIV, Hepatitis B, Tuberculosis, PLHIV, Cameroon.

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Crossref Doi: <https://doi.org/10.36437/irmhs.2021.4.1.C>

Introduction

The term infection refers to the invasion of a living organism by germs (bacteria, viruses, fungi, or parasites). Some infectious diseases require prolonged treatment together with close monitoring. This is the case of the Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV), and Tuberculosis (TB), which are among the pathologies causing a significant number of deaths worldwide. HIV is a retrovirus that attacks the immune system and is responsible for Acquired Immune Deficiency Syndrome (AIDS), which is a state of the weakened immune system leading to opportunistic infections.¹

In 2016, about 36.7 million people were infected, and over 1 million people died of an HIV-related cause worldwide.² However, sub-Saharan Africa remains the most severely affected region and it is home to nearly 70% of People Living with HIV (PLHIV).² HIV remains a public health problem in developing countries.

In 2016, 32,000 new HIV infections and 29,000 AIDS-related deaths were counted in Cameroon.² Because of similar transmission routes, there is a risk of co-infection between HIV and Hepatitis B Virus (HBV). Hepatitis B is a viral infection that attacks the liver, it can cause acute or chronic illness, leading to a decrease in spontaneous healing. Worldwide, About 257 million people are living with Hepatitis B virus infection.³ However, the

seroprevalence of this infection in Africa remains highly variable: A study carried out in Senegal recorded 14.2% in a military population and another recorded 12.6% among blood donors at the Yaoundé Central Hospital in Cameroon.^{4,5}

TB, unlike HIV and HBV which are viral infections, is a bacterial infection that takes advantage of the failure of the Immune System to establish itself. This infectious disease caused by an airborne mycobacteria is one of the 10 leading causes of death worldwide. Out of the estimated 10.4 million people who contracted the disease in 2015, about 1.8 million died from it (0.4 million of whom also had HIV) and the number of deaths from tuberculosis is estimated to be over 95%.⁶ These deaths occur most often in low-income countries. In Cameroon, the incidence and prevalence of TB still remain high, about 70/100,000 inhabitants.⁶ HIV-HBV and HIV-TB co-infections are the leading causes of morbidity and mortality among PLHIV For example, the prevalence of HIV-HBV co-infection has been estimated to be at 9.3% in Botswana, 13.6% in Ghana, and 16% at the Central Hospital in Yaoundé, Cameroon.^{7,8,9} According to the estimates of WHO, one in three deaths among people living with HIV is due to tuberculosis.⁶ For example, the prevalence of HIV/TB co-infection has been estimated to be at 30% in Congo and 26.06% in western Cameroon.^{10,11}

Despite provisions for accompanying people living with HIV, hepatitis B, and/or TB, HIV/HBV and HIV/TB co-infections continue to go unchecked in Cameroon. This may be due to the fact that access to screening and surveillance for HBV and TB in HIV-infected patients is not systematic. As a result, many people infected with HIV are unaware of their co-infection status, resulting in an increased co-morbidities. Hence the interest of this study was to determine HIV/HBV and HIV/TB co-infection among people living with HIV at the Central Hospital and the Jamot Hospital in Yaounde.

Materials and Methods: A cross-sectional hospital-based descriptive study was carried out in PLHIV seeking care at the Central hospital and Jamot hospital in Yaounde. 362 samples were collected over a period of six (6) months, from September 2017 to March 2018. The study population consisted of PLHIV at the Central Hospital and Jamot Hospital in Yaoundé.

Inclusion Criteria: Patients of ages 15 years and above regardless of gender, ethnicity or tribe were included in our study. And also volunteer participants who agreed to sign an

informed consent form after being sensitized to the nature and procedure of the study.

Exclusion Criteria: Participants without HIV were excluded from the study.

Participants in our study were enrolled during consultation at the day hospital, and also those hospitalised at the infectiology ward, support service for PLHIV in Jamot hospital, as well as those who came for routine check-ups. Socio-demographic data and information on the possible background were obtained by means of a questionnaire provided to each participant. For the diagnosis of TB, sputum was collected in sterile containers and placed in a cooler containing blocks of ice, and then transported to the Laboratory for Tuberculosis Research and Pharmacology where microscopic examination (auramine staining) was performed. The sputum was decontaminated under the fume hood by the Cetyl Pyrimidium Chloride (CPC) method. DNA extraction was performed using the GenoLyse Version 1.0 Hain Life science kit, followed by PCR amplification of the IS6110 sequence of Mycobacterium Tuberculosis (MTB) and then further made to migrate at 2% agarose gel electrophoresis.

Target gene	Primers	Primer sequence	Size (bp)	Reference
IS6110	IS6110F	5'-CCTGCGAGCGTAGGCGTCCG-3'	123	Eisenach <i>et al.</i> [12]
	IS6110R	5'-CTCGTCCAGCGCCGCTTCGG-3'		

Table 1: Characteristics of primers

For the diagnosis of HBV, 5 ml of blood was collected from all patients in a dry tube, and serum was obtained after centrifugation at

3000 turns for 5 minutes. A rapid diagnostic test (RDT) was performed using the DiaSpot HBsAg Kit to determine HBV surface antigen.

Positive results on the TDR were further confirmed by ELISA Sandwich (Kit Foresight). The data were entered using Microsoft office excel 2013 software. SPSS software version 22.1 was used for data analysis. Generally, data analysis was descriptive estimating the proportion of HIV patients co-infected with TB and HBV respectively. However, data analysis was segregated to show the burden in Male and females and also in different age and other population subgroups. For precision purposes, the confidence interval at 95% was estimated for each proportion.

Ethical Considerations: This study has received ethical approval (N°2018/01/970/CE/CNERSH/SP) from the National Ethical Committee for Human Health Research (NECHHR). It was approved institutional by the Director of the Yaounde Central Hospital and Director of the Yaounde

Jamot Hospital. Blood and sputum samples were only taken from patients who signed an informed consent form, and only the investigator had access to their data, in order to respect patient's confidentiality. Patients under the age of 21 have the parental consent form and the minor's assent form.

Results

Distribution of the target population (HIV) by age group and gender: During this study, we recruited 362 PLHIV. The minimum age was 15 and the maximum age was 80. The study shows that women were mostly affected by HIV infection, having a prevalence of 67.1% percent compared to 32.9% percent among men. The age group most affected in both males and females were those between the ages of 35-44 years (30.1%) with a prevalence of 9.9% in males and 20.2% in females (Table 2).

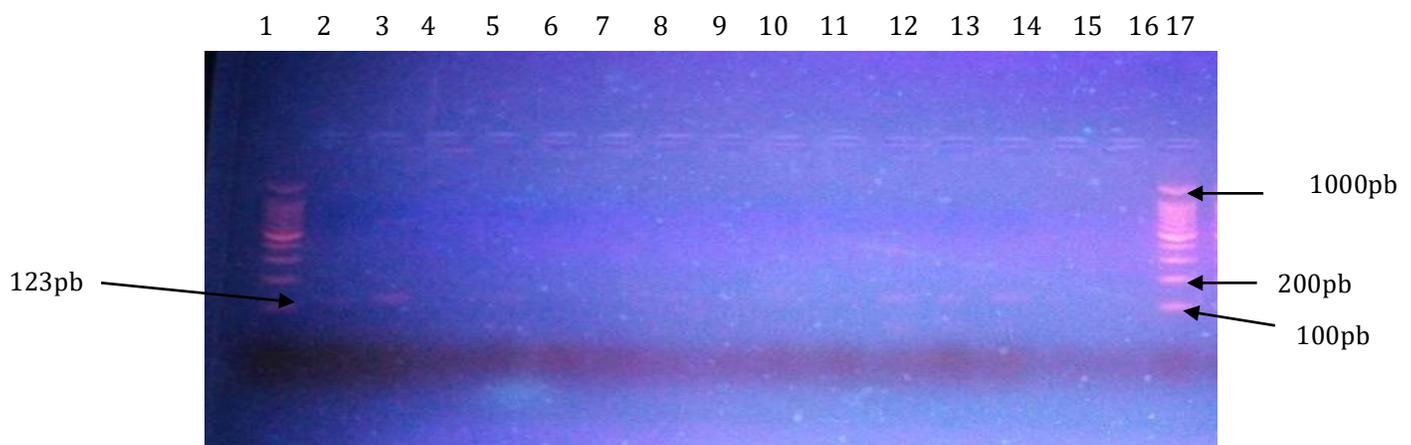
GENDER			
Age range (years)	Female	Male	Total
15 à 24	21 ^a (5.8% ^b)	9 ^a (2.5% ^b)	30 ^a (8.3% ^b)
25 à 34	68 (18,8 %)	23 (6,4 %)	91 (25,2 %)
35 à 44	73 (20,2 %)	36 (9,9 %)	109 (30,1 %)
45 à 54	49 (13,5 %)	28 (7,7 %)	77 (21,2 %)
≥ 55	32 (8,8 %)	23 (6,4 %)	55 (15,2 %)
Total	243 (67,1 %)	119 (32,9 %)	362 (100 %)

a= Number of patients, b= Percentage.

Table 2: Distribution of infection by age group and sex among HIV-positive patients

Results on microscopy and PCR for the identification of patients with tuberculosis: Out of the 362 sputum samples collected, 18 sputum samples, were positive for Mycobacterium Tuberculosis with microscopy (5%) among which we had 13 men (3.6%) and 5 women (1.4%). Note that for the further analysis using (PCR), we directly used the decontaminated sputum which was stored at +4°C. In the end, 33 sputum samples were positive for MTB with PCR (9.1%), among which we had 19 men (5.2%) and 14 women (3.9%).

Mycobacterium Tuberculosis PCR Amplification Results: After the amplification reaction, we migrated the amplicons onto the 2% agarose gel by electrophoresis. We obtained different bands corresponding to the amplification products of the loci. The size of the amplicons was determined manually from the size and migration distance of the molecular weight markers (kilobase: kb) used (1000kb). The bands were obtained at 123 Base Pairs (Pb) (Graph 1).



Graph 1: PCR Amplicon Electrophoresis.

Legends: 1 and 17: Molecular weight marker, 2: Positive control, 16: Negative control and 3-15: Samples.

Seroprevalence of HBV with RDT and ELISA: Out of the 362 PLHIV, 55 samples were positive for HBV (15.2%) with RDT against 49 samples which were positive for HBV (13.5%) with ELISA.

Prevalence of HIV/HBV/TB co-infections by gender: The prevalence of HIV/HBV co-

infection was 13.5%, HIV/TB co-infection was 9.1%, and HIV/HBV/TB triple infection was 0.9%. Females were the most affected among the HBV/HIV co-infected persons (7.7%), while males were mostly affected among the HIV/TB and HBV/TB co-infected persons with 5.2% and 0.6% respectively (Table 3).

		HIV	HIV/HBV	HIV/TB	HIV/HBV/ TB	Total
Sex	Female	200 (55,2 %)	28 (7,7 %)	14 (3,9 %)	1 (0,3 %)	243(67,1 %)
	Male	77 (21,3 %)	21 (5,8 %)	19 (5,2 %)	2(0,6%)	119 (32,9)
Total	Prevalence	277(76,5 %)	49 (13,5 %)	33 (9,1 %)	3 (0,9 %)	362 (100 %)
	Ic 95 %	(71,89-80,59) %	(10,39-17,45) %	(6,56-12,53) %	(0,28-2,41) %	

Table 3: Prevalence of co-infections by gender

Prevalence of HIV/HBV/TB co-infections by occupation: The results of this study show that labourers were mostly affected by these infections (44.5%) compared to other occupations. This is the case for HIV mono-

infected (34.3%), HBV/HIV co-infected (5.2%), HIV/TB co-infected (4.7%). In contrast, among the triple HBV/HBV/TB infected, those unemployed were those most affected (0.6%) (Table 4).

Occupations	HIV	HIV/HBV	HIV/TB	HIV/HBV/ TB	TOTAL
Students	28 (7,7 %)	7 (1,9 %)	0 (0 %)	0 (0 %)	35 (9,7 %)
Skilled labourers	49 (13,5 %)	9 (2,5 %)	7 (1,9 %)	0 (0 %)	65 (18 %)
Unskilled labourers	124 (34,3 %)	19 (5,2 %)	17 (4,7 %)	1(0,3 %)	161 (44,5 %)
Unemployed	76 (21 %)	14 (3,9 %)	9 (2,5 %)	2 (0,6 %)	101 (27,9 %)
TOTAL	277 (76,5 %)	49 (13,5 %)	33 (9,1 %)	3 (0,9 %)	362 (100 %)

Table 4: Frequency Distribution of Infections according to occupations

Prevalence of HIV/HBV/TB co-infections by marital status: The results of this study show that those who are single were mostly infected with HIV (39.5%) and HBV/HIV (8.3%) compared to those who are married.

On the other hand, patients who are married were mostly infected with HIV/TB (5.5%) and HBV/HBV/TB (0.6%) compared to those who are unmarried (Table 5).

Marital status	HIV	HBV/HIV	HIV/TB	HIV/HBV/TB	TOTAL
Single	143(39,5 %)	30 (8,3 %)	11 (3 %)	1 (0,3 %)	185(51,5 %)
Married	84(23,2 %)	14(3,9 %)	20(5,5 %)	2 (0,6 %)	120(33,1 %)
Divorced	10 (2,8 %)	5(1,4 %)	0(0 %)	0(0 %)	15 (4,1 %)
Widower	40(11 %)	0 (0 %)	2(0,6 %)	0 (0 %)	42 (11,6 %)
TOTAL	277(76,5 %)	49 (13,5 %)	33 (9,1 %)	3 (0,9 %)	362 00 %)

Table 5: Frequency distribution of Infections according to marital status

Discussion: Our results show that the mean age of the population was 40 years, indicating an increased consideration of young people in this age group for preventive measures against HIV/AIDS and related infections. The most affected age group was 35-44 years old with a percentage of 30.1%, this could be justified by the fact that this is the age group at the sexually active stage according to UNAIDS. Out of the 362 PLHIV included in this study, 243 (67.1%) were women and 119 (32.9%) were men. These results are very similar to those obtained in Cameroon, where, out of 531 PLHIV samples recruited in 5 regions of Cameroon, 68% were women and 32% were men.¹³ Similarly, in a study of 209 PLHIV in the Democratic Republic of Congo, 67.4% were women and 32.6% were men.¹⁴ The Commission on HIV/AIDS and Governance in Africa explains this feminization, which is mostly found in Sub-Saharan Africa, in relation with other biological factors such as a much longer duration of viral contact compared with men, also with the fact that women are highly exposed than men. In this study, we amplified the IS6110 sequence of *Mycobacterium tuberculosis* by PCR directly from the decontaminated sputum. PCR is a technique commonly used routinely in

laboratories to identify *M. tuberculosis* in clinical specimens. Most *M. tuberculosis* complex species have 10-15 copies of the IS6110 sequence. This feature helps to increase the sensitivity of the PCR on this amplification obtained from a single DNA sequence. After amplification of the IS6110 sequence, the 123 pb obtained here were similar to the amplicon sizes obtained from previous studies.^{15,16} This could be explained by the fact that the sputum of our patients' contained the germ responsible for tuberculosis. From the results, the PCR permitted us to identify the false-negative results from microscopy, because with PCR we obtained 9.1% of TB positive against 5% of TB positive with microscopy. This could be due to the dissemination of the bacteria in immunodeficient patients. PCR is therefore a technique of choice for the identification of MTB in patients suffering from tuberculosis because, unlike other techniques, it is more sensitive and specific.¹⁷

Serological analysis of the specimens permitted us to identify the false-positive results from RDT because with Elisa we obtained 13.5% of HBV positive against 15.2% of HBV positive with RDT. ELISA permitted us to identified 49 HBV-positive

patients out of 362 HIV-positive patients. This corresponds to an HIV-HBV co-infection seroprevalence of 13.5% [95% CI:10.39-17.45%]. The seroprevalence of HBV/HIV co-infection appears to be lower in this study compared to those obtained at the central hospital in Yaoundé among 75 PLHIV, which was 16% and in Benin 16.9% [IC95: 14.3%-19.9%].^{9,18} This could be explained by the fact that we used confirmatory tests (ELISA), unlike the other researchers who used only rapid diagnostic tests, which increases the incidence of false positives cases. This could also be due to the difference in sample size and different geographical locations. However, our results are close to that obtained in a study carried out in Ghana 13.6%.⁸ All of these results argue for a high prevalence of HBV in Sub-Saharan Africa. The rate of co-infection was significantly higher in women (7.7%) than in men (5.8%) with ($P \leq 0.05$), these results are supported by those obtained in the study carried out in Yaoundé Central Hospital where women (12%) were mostly implicated with co-infection than men (4%).^{9,19} However, men are the largest reservoir of HBV, but because of the female anatomy, women are mostly potential targets for the transmission of this infection.

The rate of HIV/TB co-infection was 9.1%, [95% CI:6.56-12.53%] this prevalence is much lower than the results obtained from some studies carried out in West Cameroon (26.6%) and Mali (30.37%).^{11,20} However, our results are closer to those obtained in the Lubumbashi health zone at 11.6%.²¹ The rate of HIV/TB co-infection was distributed as follows: 3.9% for females and 5.2% for males, these results are supported by those obtained in the Lubumbashi health zone, where men were more affected by these co-

infections than women.²² This could be explained by the predisposition of men to smoking, which would favour tuberculosis in them. These results also confirm that tuberculosis is one of the most recurrent opportunistic infection among people living with HIV/AIDS. In addition to these results concerning HIV/HBV and TB/HBV co-infections, we also had a triple infection of HIV/HBV/TB, with a prevalence of 0.9% [95% CI: 0.28-2.41%]. These results are different from those obtained in West Cameroon with a prevalence of this triple infection (HIV/HBV/TB) to be at 4.9%.²³ This could be explained by the differences in sample size.

Based on our results we can say that HIV/HBV, HIV/TB co-infections and/or triple HIV/HBV/TB infections are the main causes of mortality and co-morbidity in people living with HIV in Yaounde Cameroon.

Conclusion: At the end of our investigations, we obtained a prevalence of 13.5% for HIV/HBV co-infection, 9.1% for HIV/TB co-infection, and 0.9% for triple HIV/HBV/TB infection. Women were most affected with HIV and HIV/TB co-infection with a prevalence of 55.2% and 7.7% respectively, while men were most affected by HIV/TB and HIV/HBV/TB co-infections with a prevalence of 3.9% and 0.3% respectively. PCR is more suitable for the identification of MTB while ELISA is more suitable for the identification of HBV. It is important to diagnose HBV and tuberculosis systematically in PLHIV in order to limit the risk of antiretroviral resistance and to better manage patients living with HIV.

Availability of data and materials: The data supporting the results of this study are available in results, tables and figures of the manuscript.

Financing: This work was funded by the Central Africa Network for Tuberculosis, HIV/AIDS and Malaria (CANTAM) project.

Acknowledgements: We thank the EDCTP (European and Developing Clinical Trials Partnership) /CANTAM (Central Africa Network for Tuberculosis, AIDS/HIV and Malaria) for Two projects for the material and financial support. We thank Kouanfack Charles, Pefura Yone Walter Eric, Penlap Beng Véronique for their agreement to carry out the collection of the exchanges and some analysis in their different services and laboratory. We thank Assonfack Wamba Adonis, Kouotou Poluem Ismaila for their contribution to patient registration, blood collection, and testing; Buyohnwenda Kizito Atehambe for reviewing the manuscript.

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How to cite this Article: Kamdem Thiomo Diane, Djuidje Ngounoue Marceline, Tchinda Fossi Cedric, Kenfack Momo Raoul, Martin Ndinakie Yakum, Ambassa Axel Cyriaque, Tsasse Martine Augusta Flore, Assam Assam Jean Paul, Penlap Beng Veronique; [Prevalence of Hepatitis B and Tuberculosis among People Living with HIV \(PLHIV\) in Cameroon](#); *Int. Res. Med. Health Sci.*, 2020; (4-1): 23-33; doi: <https://doi.org/10.36437/irmhs.2021.4.1.C>

Source of Support: Nil,

Conflict of Interest: None declared.

Received: 17-12-2020; **Revision:** 5-2-2021; **Accepted:** 7-2-2021