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Sero-prevalence of Human Immunodeficiency Virus and hepatitis viruses and their correlation with CD4 T-cell lymphocyte counts in pregnant women in the Buea Health District of Cameroon

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ABSTRACT

Prior to this study, very few studies in Cameroon have addressed co-infection of HIV and hepatitis in pregnancy. The aim of this study was to screen pregnant women living in the Buea Health District for Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV) and Hepatitis C virus (HCV) and determine how the prevalence of mono and co-infections relates to immunity. Eligible and consenting women were interviewed using a standardized questionnaire. After which 10 ml of whole blood was collected from 406 pregnant women. All specimens were tested for HIV, HBV and HCV using Determine HIV1/2 (Abbott Co. Ltd., Japan), SD Bioline (Standard Diagnostics, INC., USA) and ELISA (Murex Biotech Limited, Temple Hill, UK; ABBOTT Laboratories, Wiesbaden, Germany). The prevalence of HIV, HBV and HCV mono-infection in this study was 34(8.37%), 40(12.1%) and 28(6.89%) respectively. HIV/HBV, HIV/HCV, HBV/HCV and HIV/HBV/HCV multiple infection rates were 6(1.48%), 7(1.72%), 5(1.23%) and 2(0.49%) respectively and there was no statistical association between accessed risk factors and multiple viral infections in Pregnancy. Amongst women with a positive HIV result, those co-infected with HBV and HCV had the least mean $CD4^+$ T-cell lymphocyte counts (531.50 ± 6.18) while those infected only with HIV had the highest mean $CD4^+$ T-cell lymphocyte counts (620.22 ± 165.27). Co-infection of HIV, HBV and HCV reduced the immune competence-$CD4^+$ T cell counts, in pregnant women more than those who were HIV mono-infected.

Keywords: Mono-infection, multiple-infections, mother-to-child-transmission, CD4 T-cell lymphocyte, pregnancy.

INTRODUCTION

People living with HIV/AIDS (PLWH) were 34 million (Range 31.4-39.5 million) and AIDS deaths were 1.7million (Range 1.5-1.9 million) (Schwartlander et al., 2011). In 2013, WHO reported an increase in these

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figures stating that by the end of 2012, PLWH had increased to 35.3 million and 1.6 AIDS death occurred (WHO-HIV/AIDS Report, 2013). Due to the high estimated numbers of pregnant women living with HIV, Cameroon is one of 22 priority countries identified in the World Health Organization's "Global Plan" as a key target for HIV control and interventions (Gaynes et al., 2012).

Pregnancy is considered high risk when a woman is hepatitis B virus (HBV) infected and there is a 20% chance of transmission to the unborn infant. HBV is the most common form of Hepatitis worldwide and chronic carriers continue to transmit the virus years before symptoms appear. The mode of transmission in areas of high endemicity is mostly vertical (Tran, 2010; Hwang and Cheung, 2011; Komatsu et al., 2012). Of the hepatotrophic viruses (hepatitis A, B, C, D and E), hepatitis B virus is most virulent and most versatile and is probably also the most prevalent of all viruses that affect humans (Park, 2011). In a study carried out in Ethiopia, it was found that the sero-prevalence of HBsAg in pregnant women was 3.7% (Awole and Gebre-Selassie, 2005; Esan et al., 2014; Molla et al., 2015).

In Cameroon, studies carried out revealed that the prevalence of hepatitis B surface antigen in rural general populations was greater than 8% (Kfutwah et al, 2012). Presently, much of the work being done in Cameroon on HBV is focused on hepatitis among HIV patients and in one of such studies, the prevalence of HBV among adults starting ARV therapy was found to be 8.3% (Laurent et al., 2010).In the North West Region of Cameroon, it was determined that the prevalence of hepatitis B surface antigen in the general population was at 12.6% and male patients were more likely to have positive results than female patients (Zoufaly et al., 2011). More still has to be done in the South West Region of Cameroon as little data is available concerning the prevalence of HBsAg and hepatitis B virus (HBV) infection may go undetected. Unawareness of an ongoing infection delays the diagnosis of HBV-related liver disease and favors the spread of the virus (Chan et al., 2012; Brouard et al., 2013; Molla et al., 2015).

In a study to demonstrate unawareness of HBV infection among patients in a clinic in Italy by Ippolito et al. (2011), it was noticed that up to 40.3% of the participants were not aware of their infection. The age group most likely to be affected around the world is the newborn population, particularly in areas with a high prevalence of disease and lack of identification of infected women whose infants are at risk for becoming chronic carriers and in regions with low spread perinatal screening and in- adequate use of newborn prophylaxis, horizontal transmission secondary to exposure to contaminated blood products, body fluids, or sexual contact become the primary modes of transmission of HBV in the young adult population (Komatsu et al., 2012).

Hepatitis C virus (HCV) which is also being reported is an RNA virus known to infect humans and chimpanzees, causing similar disease in these 2 species. HCV is most often transmitted parenterally but is also transmitted vertically and sexually (Karoney and Silika, 2013). Hepatitis C virus (HCV) is up to 4 times more infectious than HIV and it is the leading cause of chronic liver cancer (Karoney and Silika, 2013). Drops of the virus can get dry and remain infectious for 6 weeks at room temperature. It also requires less exposure than HIV to cause infection (Te and Jensen, 2010). The prevalence of HCV in the general population in Africa ranges between 0.1% and 17.5%, depending on the country (Karoney and Silika, 2013). Little data is available on HCV morbidity and mortality in Africa. Of those HCV-infected patients who develop chronic liver disease 1.6% progress to Hepatocellular carcinoma (HCC), a condition

with a mortality rate >80% (Maheshwari and Thuluvath, 2010).

The prevalence of HCV infection in Cameroon is among the highest in the world, 7.9% as reported by Njouom et al. (2005) and comparable to that documented in the schistosomiasis-endemic areas of Egypt (Frank et al., 2000). Just like in Egypt, the high prevalence of HCV has been incriminated to interventions for tropical disease control during the colonial era (Jacques et al., 2010) and the use of unsterilized pricking equipment used for mass treatment on the general population with parenteral anti-schistosomal therapy from the 1920s to the 1980s or traditional practices (Mohamoud et al., 2013). Despite its high prevalence and highly infectious nature, HCV remains under-diagnosed and under reported in Africa (with the exception of Egypt). Low risk of transmission of HCV from mother to child has been reported in Cameroon (Pépin et al., 2010; Njouom et al., 2011).

All the three viruses have some common areas of interactions such as; shared routes of transmission- in blood either sexually or by injection drug use and mother-to-child-transmission (M-T-C). HBV like HIV possess reverse transcriptase (Mims et al., 2006) and 5-25% of the 35.3 million people living with HIV worldwide are co-infected with either HBV or HCV or both (Okeke et al., 2012). The highest numbers of those co-infected are in low income countries (Okeke et al., 2012).

This study was carried out because previous studies on HIV and hepatitis co-infection in Cameroon have been on the general population and 90% of HBV transmissions are vertical with more women transmitting the Hepatitis B surface antigen (HBsAg). Also, it was to provide relevant data on current prevalence of HIV and hepatitis co-infection and their correlation to immunity ($CD4^+$ T-cell counts) in pregnant women in the Buea Health District, South West Region of Cameroon.

MATERIALS AND METHODS

Ethical considerations

Ethical approval for this work was obtained from the Regional Delegation of Public Health, Buea, South-West Region and from the Ministry of Public Health, Yaounde, Cameroon. Participation was voluntary. A written consent was sought from all participants. All procedures were standard and only involved minimal risk to the participants.

Study setting/Study population/ Eligibility criteria

Study participants were recruited from Antenatal Clinics (ANCs) in the Buea Health District of Cameroon. A total of 407 participants were interviewed and criteria for recruitment were being pregnant and willing to participate in the study.

Sample collection

This was a demographic cohort study carried out in 2010. All eligible and consenting women were interviewed using a standardized questionnaire. After interview, the following samples were collected from each participant: 10 ml of whole blood (5 ml was put in dry tubes to obtain serum while the remaining 5 ml was put in EDTA tubes to obtain plasma). The blood samples collected were put in tube racks and transported in an ice-box containing ice to the Faculty of Health Science Laboratory, where all laboratory assays were done. Participants' results were returned and those of HIV-positive women after post-test counselling were directed into regular care by the nurses in the antenatal clinics. Two and a half milliliter (2.5 ml) of whole blood was equally collected from children born of HIV positive mothers 18 months after delivery to test for HIV.

Sample analysis

All specimens were tested for HIV, HBV and HCV using Determine HIV1/2 (Abbott Co. Ltd., Japan), SD Bioline (Standard Diagnostics, INC., USA) and ELISA (Murex Biotech Limited, Temple Hill, UK; ABBOTT Laboratories, Wiesbaden, Germany).The $CD4^+$T-cell absolute count was determined using the Partec Cyflow Counter and the CD4 easy count kit (Partec GmbH, Germany).

Statistical analysis

Data was entered and checked for completeness using excel and analyzed using SPSS. Differences between groups were established for continuous data using the student t-test and the analysis of variance (ANOVA) while the Chi-square (X^2) analysis was used to establish difference between groups for categorical data. The correlation coefficient was calculated to establish a correlation between two variables. A P- value < 0.05 was considered statistically significant.

RESULTS

Description of participants' characteristics

A total of 407 pregnant women were approached in the study but 406 consented and this gave a response rate of 95.54%. Slightly less than one third (30.5%) of the participants were primiparous. The age of participants ranged from 15 to 47 years with a mean of 26 ± 5.56 (Table 1).

Prevalence of HIV, HBV and HCV mono and multiple infections

Twenty (4.93%) of the 406 pregnant women tested positive for two or all three infections (HIV, HBV and HCV) screened in this study. Six [1.48% (P=0.297)] were found to be co-infected with HIV and HBV, 07 [1.72% (P=0.135)] with HIV and HCV, 05 [1.23% (P=0.30)] with HCV and HBV, and 2 [0.49% (P=0.293)] were infected with all three infections as illustrated in Figure 1. These results were not statistically significant and none of the risk factors were associated with multiple infections as illustrated on Table 2.

Mother–to-Child HIV transmission amongst HIV positive pregnant women

Of the 34 HIV infected pregnant women followed up in this study, 32 (94.12%) gave birth to life children, and 3 (8.82%) were lost to follow up, giving a successful follow up rate of 29 (85.29%). Amongst the 29 children born of HIV positive mothers, 8 (27.59%) were diagnosed to be HIV positive.

The mean $CD4^+$T-cell count of the 29 mothers who gave birth to life children was 251.57 ± 80.33 (95% CI: 221.01-282.13) while that of mothers who transmitted the virus to their children was 202.1± 46.01 (95% CI: 163.66-240.59).

The impact of HBV and HCV co-infection on the immune competence of HIV positive pregnant women

Amongst women with a positive HIV result, those co-infected with HBV and HCV had the least mean $CD4^+$ T-cell lymphocyte counts (531.50 ± 6.18) while those infected only with HIV had the highest mean $CD4^+$ T-cell lymphocyte counts (620.22 ± 165.27) and there was a significant difference between the $CD4^+$ T-cell lymphocyte counts of HIV positive only and HIV negative pregnant women (P= 0.002). Amongst those who had a positive test result for at least one viral infection, those with a positive test result for both HBV and HCV had the highest mean $CD4^+$ T-cell lymphocyte counts (843.00 ± 223.54) and there was no significant difference between the $CD4^+$ T-cell lymphocyte counts of HCV positive and negative pregnant women (P= 0.602) as illustrated in Figure 2.

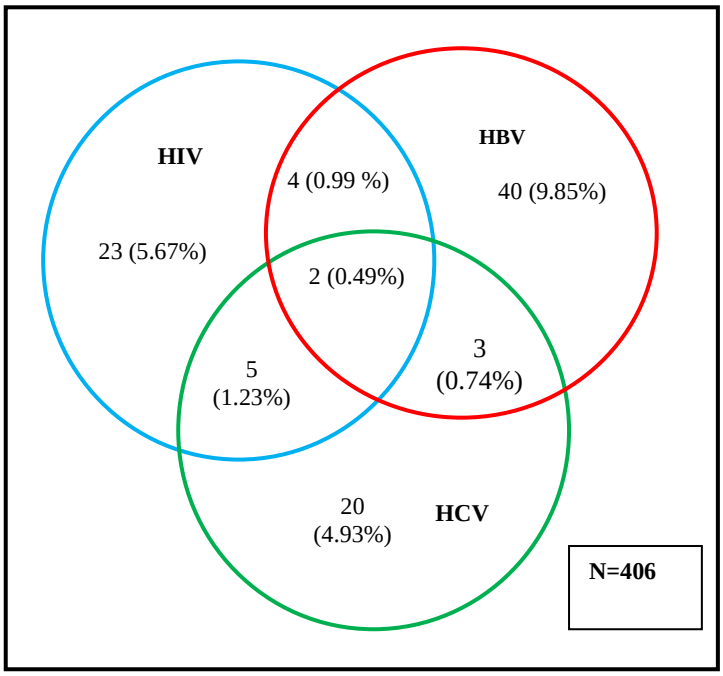

Figure 1: Distribution of HIV, HBV and HCV multiple infections amongst study population.

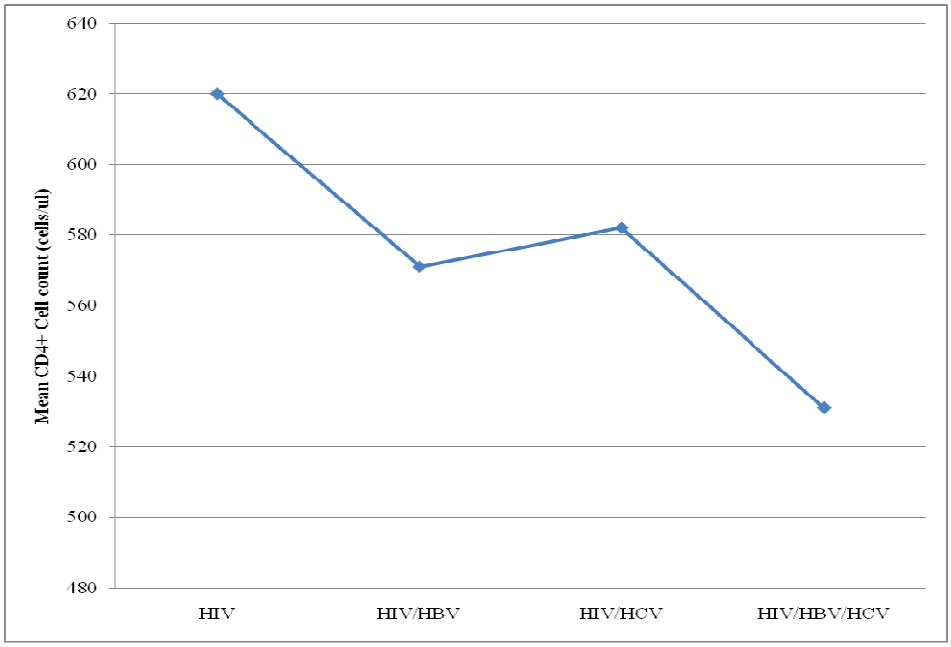

Figure 2: Distribution of Mean $CD4^{+}$ T-cell lymphocyte counts by Health Condition.

Table 1: Distribution of Participants' Characteristics.

Age group	Frequency	Percentages	Cumulative Percentages
≤25 years	187	46.1	46.1
≥ 26 years	219	53.9	100.0
Marital status			
Unmarried	144	35.5	35.5
Married	256	63.1	98.5
Widow/divorced	6	1.5	100.0
Period of gestation			
1st trimester	30	7.4	7.4
2nd trimester	160	39.4	46.8
3rd trimester	216	53.2	100.0
Number of gravidity			
1	141	34.7	34.9
2	102	25.1	60.1
3	79	19.5	79.7
4	49	12.1	91.8
5	22	5.4	97.3
6	7	1.7	99.0
7	2	0.5	99.5
8	2	0.5	100.0
Education			
No formal education	3	0.7	0.7
Primary	119	29.3	30.0
Secondary	178	43.8	73.9
Tertiary	106	26.1	100.0
Occupation			
Semi-skilled	176	43.3	43.3
Skilled	86	21.2	64.5
Student/apprentice	80	19.7	84.2

Table 2: Risk factors to multiple viral infections in pregnancy.

Risk factors	**HIV/HBV**		**p-Valve**	**HIV/HCV**		**p-Valve**	**HBV/HCV**		**p-Valve**	**HIV/HBV/HCV**		**p-Valve**
Age Group	**Neg**	**Pos**		**Neg**	**Pos**		**Neg**	**Pos**		**Neg**	**Pos**	
≤ 25	185	2	0.529	185	2	0.349	185	2	0.784	186	1	0.911
≥26	215	4		214	5		216	3		218	1	
Marital status												
Unmarried	140	4	0.271	141	3	0.254	142	2	0.946	142	2	0.161
Married	254	2		252	4		253	3		256	0	
Widow/Divorce	6	0		6	0		6	0		6	0	
Educational level												
No formal education	3	0	0.727	3	0	0.369	3	0	0.533	3	0	0.462
Primary	118	1		117	2		119	0		119	0	
Secondary	174	4		173	5		175	3		176	2	
Tertiary	105	1		106	0		104	2		106	0	
Occupation												
Unskilled	173	3	0.473	172	4	0.454	175	1	0.673	175	1	0.506
Skilled	85	1		85	1		84	2		86	0	
Student/apprentice	80	0		80	0		79	1		80	0	
House wife/Unemployed	62	2		62	2		63	1		63	1	

DISCUSSION

Cameroon is part of Sub-Saharan Africa, a region where 68% of all people living with HIV reside (UNAIDS, 2013). It is estimated that 550,000 of the world's 35.3 million people are currently living with HIV in Cameroon (WHO-HIV/AIDS, 2013) with an HIV prevalence of 5.5% (Gaynes et al., 2012). The South West Region of Cameroon represents one of the high HIV burden regions with an estimated 8% prevalence (Ndjeng, 2013). This study reveals an HIV prevalence of 8.4% in pregnant women attending antenatal clinics the Buea.

The prevalence of HBsAg among pregnant women was found to be 12.1%. This is relatively high in view of the fact that a vast majority of participants were asymptomatic. In comparison with studies from other parts of the same country, the prevalence reported in this study was higher than the 1.2%, 7.85%, 7.7% and 9.7% reported by Thumamo and Asoquo (2004), Kfutwah et al. (2012), Fomulu et al. (2013) and Frambo et al. (2014) respectively. This difference may be because of different socioeconomic status and the natural difference attached to different geographic zones. Our prevalence was also higher than the 5.4% reported in the early nineties by Ndumbe et al. (1994) in rural pregnant women in Cameroon. We however had similar results with Florent et al. (2012), who found a prevalence of 12.14% amongst blood donors at the Blood Bank of the Central Hospital, Yaoundé (Cameroon). Similar results were also recorded by Zoufaly et al. (2012) who found a prevalence of 12.6% among HIV patients initiating antiretroviral therapy in the North-West Region of Cameroon. We however expected this high prevalence and this is in conformity with the established fact that HBsAg is endemic in African countries south of the Sahara desert of which Cameroon is among (Ndumbe et al., 1994). This level of carrier state in women of reproductive age will suggest that there is a high risk of mother-to-infant transmission in the Buea Health District. However, HBsAg carrier state alone in a pregnant woman does not put the fetus at a huge risk of being infected, instead, pregnant women who are positive both for HBsAg and HBeAg are more likely to infect their babies. Transmission does not only depend on HBsAg and HBeAg status but also on anti-HBeAg status. Anti-HBeAg positivity tends to prevent transmission (Okada et al., 1976; Umar et al., 2013).

Africa has the highest WHO estimated regional HCV prevalence (5.3%). The prevalence of HCV in the general population in Africa ranges between 0.1% and 17.5%, depending on the country (Karoney and Silika, 2013). The prevalence of HCV in this study is 6.9%. Many studies have shown that the prevalence of HCV increase with Age. Participants in our study had ages that ranged from 15 to 47 years with a mean age of 26 ± 5.56. Though there was no statistically significant difference between those aged less than or equal to 25 years and those greater than 25 years in this study, we however noticed that those with ages greater than 25 years were more infected than those less than 25 years.

The overall hepatitis (B and C) and HIV co-infection rate in this study was 4.9%. This implies that one in every twenty pregnant women is at risk of transmitting both HIV and HBV or HCV, a deadly combination for which a real cure does not yet exist, not only to their spouse but to their future offspring. Appropriate measures need to be taken to reduce the spread of these infections. Although it is postulated that trans-placenta HCV transmission is very rare, the risk of mother to child HCV transmission remains very high as the child may become infected either at delivery or by the intimate contact between mother and child (Murad et al., 2013).

Lower prevalence rates for the co-infection of HIV-HBV and HIV-HCV were recorded by Florent et al. (2012) amongst blood donors in the Yaoundé Central Hospital blood bank. Higher prevalence of HIV-HBV and HIV-HCV were however obtained by Laurent et al. (2010) and Florent et al. (2012) in Yaounde. Indeed, Laurent et al. (2010)

found among HIV-positive patients, 8.3% and 12.4% co-infections with HBV and HCV, respectively. But these results were obtained from 169 HIV-positive people eligible for antiretroviral therapy, thus at a more advanced stage of the HIV infection.

In this study, the prevalence of Mother to child transmission of HIV is 27.6%. In well-resourced health care systems, such as those in the United States, there has been dramatic progress in reducing mother to child transmission of HIV infection. Early identification of HIV infection in pregnant women through routine antenatal HIV testing; immediate assessment of HIV-infected pregnant women for their need for treatment for their own health; and provision of antiretroviral treatment when needed or antiretroviral prophylaxis if therapy is not yet required has substantially reduced the risk of infection among infants during pregnancy and delivery. When combined with elective caesarean delivery and complete avoidance of breastfeeding, these interventions have reduced the risk of HIV transmission to 1%–2% (Townsend et al., 2008).

Our study shows that all eight (100%) HIV infected children born of HIV infected mothers where born through the vaginal route. 87.5% of these pregnancies where not planned and their mothers were not on any ART. This proves the non-application of WHO recommendations on measures to prevent mother to child HIV transmission in most resource limited countries. Since 2004, WHO guidelines recommended the use of more efficacious multidrug antiretroviral regimens for the treatment and prevention of mother to child HIV transmission. These measures also included the use of highly active antiretroviral therapy for women with advanced disease and short course dual prophylaxis for healthier women not yet eligible for treatment (WHO, 2009).

The application of WHO recommendations remains a major challenged in resource limited countries such as Cameroon for many reasons such as maintaining adequate supplies of HIV tests kits and medications, a shortage of maternal and child health staff, the reliance on medical doctors for antiretroviral treatment initiation and the weak linkages between HIV diagnosis in pregnancy and antiretroviral therapy services, which often preclude women from being fast-tracked into HIV care and treatment programs. As a result of this limited capacity, many countries are unable to adapt their existing health systems according to World Health Organization preventing mother to child HIV transmission guidelines, which are amended as new evidence becomes available and more cost-effective in the long-term. Therefore, many health services are not providing HIV-infected women with the most effective drugs. One example of this is the use of single-dose nevirapine, an antiretroviral drug which, despite no longer being recommended by the WHO, in 2011 is still being used in many countries for preventing mother to child HIV transmission including Cameroon.

Findings from our study also revealed that the mean $CD4^+$T cell count of the mother who failed to transmit HIV to their children was 503.14 ± 160.665 (95% CI: 442.02,564.25) while that of mothers who transmitted the virus to their children was 392.38 ± 186.61 (95% CI: 236.36, 548.39). Our study revealed that women who were tested positive for HIV, HBV and HCV triple infection had lower $CD4^+$ cell counts than those who were either co-infected with HIV/HCV or HIV/HBV and even those who tested positive for only HIV infection. These findings were similar to those of Olufemi et al. (2009) in Nigeria who demonstrated that the mean CD4+ T cell counts of those who test positive HIV/HCV and HIV/HBV co-infections were lower than those who tested positive for HIV mono infection. However, studies carried out by Yitayih et al. (2013) showed that there was no statistically significant CD4 count mean difference between HIV mono-infected, HIV/HBV and HIV/HCV co-infected study participants. These controversial results may be partly due to the differences in the immune status of the

study participants or it may be due to the viral hepatitis. A study by Hooja et al. (2015) also proved that HIV infection may accelerate the progression of hepatic complications.

We also wish to acknowledge that our study failed to consider some factors such as duration of antiretroviral therapy for those already on antiretroviral therapy, assess if the participants who were infected with HCV and HBV were acute or chronic carriers. All of these factors may have an indirect effect on the $CD4^+$T-cell counts of our study participants.

Conclusion

The prevalence of HIV, HBV and HCV mono infections in pregnant women living in the Buea, South West Region of Cameroon remains relatively higher than most other areas in which similar studies were carried out in Cameroon. The co-infection of HIV, HBV and HCV in pregnant women in this study was however lower than in the general population and multiple infection of HIV, HBV and HCV reduced the immune competence ($CD4^+$ T-cell counts) in pregnant women more than those who were HIV mono-infected.

Over a quarter of Children born of HIV positive pregnant women were tested positive for HIV. The non-application of WHO (2009) measures to prevent mother to child HIV transmission such as the use of antiretroviral treatment when needed or antiretroviral prophylaxis if therapy is not yet required, programmed pregnancies, elective caesarean delivery and complete avoidance of breastfeeding have been incriminated as main factors associated with mother to child HIV transmission in this study.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

RE-T was the principal investigator and data collector. ESN assisted in the data collection. PT did the data analysis. HLK and TN-A designed and supervised the work. All the authors read and approved the final copy of the manuscript.

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