Research Article

Importance of biochemical analysis of the liver function in the management of disease progression in people living with HIV/AIDS and co-infected by HIV and hepatitis B virus in Cameroon

AMBASSA Axel Cyriaque[1], DJUIDJE Ngounoue Marceline[1,2], GUIATEU Tamo Ida Marlene[1], MOUNDIPA Fewou Paul[1]

[1]Department of Biochemistry, Faculty of Science, University of Yaoundé I, Cameroon

[2]«Comité d'Ethique de la Recherche et de la Santé en Afrique Centrale (CERSAC) »

Corresponding author's e-mail: axel.ambassa@yahoo.fr

ABSTRACT

Liver diseases in HIV infected persons can occur due to hepatitis B virus (HBV) and hepatitis C virus (HCV) co-infections, chronic alcoholism, and hepatic tuberculosis as well as antiretroviral drugs. Co-infection by HIV and HBV is frequently encountered with negative impact on HIV progression. The aim of this study was to evaluate the importance of biochemical analysis of the liver function in the management of disease progression in people living with HIV/AIDS and co-infected by hepatitis B virus in Cameroon. Serum of 75 patients positive for HIV was screened for HBsAg by immunochromatographic test in Yaoundé central hospital, from November 2015 to February 2016. Liver enzymes (AST, ALT, CB, TB and γ-GT), as well as CD4 T cell level determination were assessed following the standard procedures. A second blood sample was taken from HIV mono-infected and HIV/HBV co-infected after three months. The socio-demographic data was also collected. The data was entered and analyzed using SPSS version 22.1 statistical software and $p<0.05$ was considered as statistically significant. Hepatitis B virus surface antigen (HBsAg) was identified in 12 patients out of 75 HIV-positive patients for a HIV/HBV co-infection prevalence of 16%. Study participants with HIV/HBV co-infection have a high ALT mean level ($p<0.05$), than HIV mono-infected participants and the difference was statistically significant. Analysis using the second blood sample carried out 3 months later, showed significant elevation of AST, ALT, ALP, conjugated bilirubin and total bilirubin, (46.66±33.42 IU; 148.48±40.65 IU; 153.68±65.82 IU; 0.149±0.028 mg/dl; 0.75±0.089 mg/dl), while the γ-GT remained stable over time. No statistically significant CD4 count mean difference was observed between HIV mono-infected and HIV/HBV co-infected participants. The first blood sample showed significant elevation of ALT in HIV/HBV co-infected patients in the Yaoundé Central hospital. High levels of liver enzymes were seen in co-infection during the second blood sampling, hence there is a necessity of careful monitoring of these patients for better care.

Keywords: co-infection, HBV, HIV, CD4 T cells, liver enzymes

RÉSUMÉ

Les maladies hépatiques chez les personnes infectées par le VIH peuvent être dues soient aux co-infections par le Virus de l'hépatique (VHB) ou le Virus de l'hépatite C (VHC), soit à l'alcoolisme chronique, soit à la tuberculose hépatique comme médicaments antirétroviraux. Les co-infections par le VIH et le VHB sont fréquemment rencontrées avec un impact négatif sur la progression du VIH. Le but de cette étude était d'évaluer l'importance des analyses biochimiques de la fonction hépatique dans le contrôle de la progression de la maladie chez les personnes vivant avec le VIH et co-infectées par Virus de l'hépatite B au Cameroun. Les sérums de 75 patients positifs au VIH ont été prélevés pour la détermination de l'antigène de surface du Virus de l'hépatite B (AgHBs) par le test immunochromatographique à l'Hôpital Central de Yaoundé, de Novembre 2015 à Février 2016. Les enzymes hépatiques (ASAT, ALAT, BC, BT, et ɣ-GT), ainsi que les taux de CD4 ont été déterminés suivant les procédures standards. Un second prélèvement sanguin avait été fait chez les patients mono-infectés VIH et co-infectés par le VIH et le VHB après une période de trois mois. Les données sociodémographiques ont été aussi collectées. Les données ont été entrées et analysées par le logiciel SPSS version 22.1 et en recherchant la plus petite différence significative avec p d" 0,05. L'Antigène de Surface de l'Hépatite B (AgHBs) a été identifié chez 12 patients parmi les 75 patients VIH positifs, ce qui correspond à une prévalence de co-infection VIH/VHB de 16%. Les patients co-infectés par le VIH/VHB avaient une élévation significative de l'ALAT (pd" 0,05), par rapport aux patients mono-infectés VIH et la différence était statistiquement significative. Les analyses du second prélèvement effectués après trois ont montré une élévation significative d'ASAT, ALAT, PAL, Bilirubine Conjuguée et Bilirubine Totale, (46,66±33,42 IU; 148,48±40,65 IU; 153,68±65,82 IU; 0,149±0,028 mg/dl; 0,75±0,089 mg/dl), tan disque la ɣGT est restée stable au cours du temps. Aucune différence significative entre les taux de CD4 n'a été observée entre les patients mono-infectés par le VIH et co-infectés par le VIH/VHB. Le premier prélèvement a montré une élévation significative d'ALAT chez les patients co-infectés à l'hôpital Central de Yaoundé. Le second prélèvement a montré une élévation signification des enzymes chez les patients co-infectés, ce qui interpelle à un meilleur suivi de ces patients.

Mots clés : co-infection, VIH, VHB, Taux de CD4, enzymes hépatiques

INTRODUCTION

Diseases of the hepatobiliary system are a major problem in patients with Human Immunodeficiency Virus (HIV) infection. An estimated one-third of deaths in HIV patients are directly or indirectly related to liver disease. Liver diseases in HIV infected persons can occur due to hepatitis B virus (HBV) co-infections, chronic alcoholism, and hepatic tuberculosis or due to hepatitis caused by antiretroviral drugs. Main routes of HIV transmission are similar to that followed by hepatotropic viruses (HBV, HCB). As a consequence, infection with HBV is an important hallmark in HIV infected patients. While HIV-HCV co-infection has predominantly been associated with non-sexual parenteral route of transmission, the HIV-HBV co-infection has been linked to both intravenous injection and sexual route of transmission (Swati and Sarman, 2006). There are estimated 36.9 million people living with HIV/AIDS worldwide and nearly 240 million are chronic HBV carriers (WHO, 2015), (OMSHEPATITE, 2015), and sub-Saharan Africa remains the region most affected by the global Acquired Immunodeficiency Syndrome (AIDS) pandemic. (Diop-Ndiaye et al., 2008). On the other hand Hepatitis B virus (HBV) constitutes a major public health challenge in this same region of the world with prevalence of >8% of the population. Epidemiologically, HIV and HBV have common routes of transmission (Vincent et al., 2005), hence the frequent occurrence of their co-infections. HIV infection negatively impacts HBV infections by decreasing the rate of Hepatitis B surface antigen (HBsAg) clearance (Dieterich, 2007). HIV-HBV co-infection negatively affects the liver function. A raised serum ALT, AST reflects direct hepatocellular damage or liver dysfunction (Pratt and Kaplan, 2000). Liver enzymes AST, ALT ALP levels have been reported by Obi et al (Ballah et al., 2012) to be significantly higher in co-infection with hepatotropic viruses compared with mono-infection and control group under antiretroviral therapy (Otegbayo et al., 2008) in south western Nigeria had similar results; (Ballah et al., 2012) in the same environment and in Eastern part of Nigeria (Ibeh et al., 2013). In Cameroon, liver disease caused by chronic hepatitis B virus (HBV) is emerging as a significant cause of morbidity and mortality among human immunodeficiency virus (HIV)-infected individuals. In addition, there is no report about liver enzyme levels among HIV mono-infected and HIV-HBV co-infected patients in Cameroon. Therefore, the aim of this study was to show the importance of biochemical analysis of the liver function in the management of disease progression in people living with HIV/AIDS and co-infected by HIV and hepatitis B virus, attending the Yaoundé Central Hospital in Cameroon.

MATERIALS AND METHODS

Settings

Biological material (serum, plasma) and equipment was used in this study. This was a prospective and analytical study from November 2015 to February 2016, at the Yaoundé Central Hospital.

Sampling

The study population consisted of patients who were in consultation and observation in the Yaoundé Day Care Central Hospital. A total of 75 HIV-positive patients were enrolled, plasma and serum were collected and stored and directly analyzed, or stored at -20°C for subsequent analysis.

Participants in this study were aged 21-49 regardless of gender, ethnicity or tribe. The volunteers, participants who agreed to sign an informed consent form after being informed of the nature of the procedure of the study, potential benefits and potential risks were recruited. For Minor participants aged 15 to 20 years, informed consent was given by their parents or guardians together with minor's assent. Patients with a history of jaundice were excluded from this study.

Date collection procedure

Socio-demographic information and other relevant possible risk factors of the study participants were collected using structured and pre tested questionnaire by trained nurses and physicians. Ten milliliter (10 ml) of venous blood was aseptically collected using plain and EDTA vacutainer tube (5 ml in each tube) for the determination of HBV seroprevalence, CD4 count and liver enzyme levels from each study participants. The blood specimen in the plain tube was centrifuged at 3000 RPM for 5 minutes to separate the serum and used for determination of liver enzyme levels within one hour of separation. The remaining serum kept in deep refrigerator ("20°C) until detection of HBV.

Statistical analysis

Data were subjected to statistical analysis for medium and significant differences using SPSS 22.1 and Excel 2010 (graphics). (Analysis of Ordered Variables (ANOVA) used as statistical tests to a factor and applying the t-test Student Newman Keuls (parametric) and seeking the significant difference with p d" 0.05.). The statistical tests used were x2.

Ethical considerations

The study received an ethical clearance from the Cameroon National Research Ethics Committee for Human Health. N° 2015/11 /665/CNERSH /SP. In addition, informed consent of participants was obtained prior to their enrollment.

RESULTS

Socio-demographic characteristics

In the present study, serum of 75 patients positive for anti HIV 1& 2 antibodies was tested for hepatitis B surface antigen (HBsAg). A total of 12 patients were found to be positive for HBsAg i.e. the prevalence rate was found to be 16 %. Out of these 12 patients, 3(4%) were male and 9(12%) were female. The average age was 36 years. The sex ratio male-female was 1: 3 60 (78. 65%) patients were aged between 21 and 49.

Mean CD4 and liver enzyme levels and their association with HIV mono-infection and HIV/HBV co-infection in the first blood sample.

The mean serum AST, ALT, ALP, TB and Conjugated bilirubin in HIV mono-infected study participants were 28.20±16,06 International Unit (IU), 28.20±16.06, 20.61±17.38IU, 100.63±48.99IU, 0.31±0.57 mg/dL and 0.014±0.011mg/dL respectively. However, in HIV-HBV co-infected study participants, only the increase of ALT activity were obtained (36.10±45.55 IU), the levels of AST, ALP, TB and CB were non-significantly raised (28.20±16.06IU, 92.51±25.91IU, 0.33±0.39m/ dL 0.018±0.016mg/dL). The mean CD4 count in HIV mono-infected patients were 336.32±239.31cells/mm^3 and the mean CD4 count in HIV/HBV co-infected were 353.08±229.30 cells/mm^3 respectively. However, the difference was not statically significant (**table 1).**

Table 1: Mean CD4 and liver enzyme levels and their association with HIV mono-infection and HIV/HBV co-infection

Type of infection	HIV Mono-infection	HIV/HBV Co-infection	P value
Size	63 (84.0%)	12 (16.0%)	$X^2 = 34.68$ (P < 0,0001*)
AST (IU)	28.20±16,06	28.74±16,84	P = 0.924
ALT (IU)	20.61±17,38	36.10±45,55	P = 0.050*
ALP (IU)	100.63±48,99	92.51±25.91	P = 0.607
CB (mg/dL)	0.014±0,011	0.018±0,016	P = 0.269
TB (mg/dL)	0.31±0.57	0.33±0.39	P = 0.924
Rate of CD4(cells/mm^3)	336.32±239.31	353.08±229.30	P = 0.818

Mean liver enzyme levels and their association with HIV mono-infection and HIV/HBV co-infection in the second blood sample

After three months, the results showed significant elevation of AST, ALT, ALP, conjugated bilirubin and total bilirubin ($p<0.05$), in HIV-HBV co- infected patients (**table 2**).

Table 2: Mean liver enzyme levels and their association with HIV mono-infection and HIV/HBV co-infection

Type of infection	HIV Mono-infection	HIV/HBV Co-infection
Size	63 (84.0%)	12 (16.0%)
AST (IU)	26.87±24.35	46.68±40.55
ALT (IU)	25.21±20.35	148.48±33.42
ALP (IU)	132.98±44.44	153.68±65.82
CB (mg/dL)	0.149±0.028	0.149±0.028
TB (mg/dL)	0.76±0.17	0.75±0.08

DISCUSSION

In this study, HIV/HBV co-infection rate was 16% which is more or less comparable with 12.5% and 12.6% prevalence (Laurent et al. 2010), (Zoufaly et al., 2012), among HIV-1 infected Cameroonian adults initiating antiretroviral therapy. This prevalence is comparable to studies from Senegal 16.8% (Diop-Ndiaye et al., 2008), and Uganda 17% (Ochola et al., 2013), however, the present prevalence was lower as compared to studies reported in Ghana (37%) (Olawumi et al. 2014), and Malawi (20.4%) (Nyirenda et al., 2008). In the present study the prevalence of co-infection was higher in females than in males (12% vs. 4%). The difference was statistically significant ($P = 0.05$). This finding is comparable with studies from Uganda (Ochola et al., 2013). This trend can be explained on the basis of higher rate of sexual promiscuity and other exposure risks in females. The major risk factor was heterosexual accounting for 92.9% of patients. This is in concurrence with other studies from India. (Gupta and Singhi, 2010), (Saravanan et al., 2007).

In the present study, there is no statistically significant CD4 count mean difference between HIV mono-infected and HIV/HBV co-infected study participants. However, study participants who had HIV/HBV co-infection in this study have the mean CD4 count (353.08±229.30 cells/mm^3) which was incomparable with mean CD4 count of 141.6 cells/mm^3 and 121 cells/mm^3 in the studies which were conducted in South Africa and Nigeria respectively (Odenyo et al., 2000), (Olufemi et al., 2009). These controversial results may be due to the differences in the immune status of the individual who participated in the study or it may be due to the viral hepatitis. In individuals who have both HIV and HBV infections, there may be high HIV and HBV viral replication that may further contribute to the impairment of the immune system of the patients. This study compared total bilirubin, conjugated bilirubin, alkaline phosphatase (ALP), aspartate amino transaminase (AST) and alanine amino transaminase (ALT) levels in HIV/HBV co-infected patients with HIV mono-infected patients. Baseline ALT was significantly higher among HIV/HBV co-infected participants compared to those with HIV alone and this is in agreement with the findings of other investigators (Zhou et al., 2007), Zoufaly et al., 2012). The ALT is found in serum and in various bodily tissues, but is most commonly associated with the liver. A raised mean serum ALT concentration

above the acceptable range principally reflects direct hepatocellular damage or liver dysfunction (Pratt and Kaplan, 2000).

No significant variation of biochemical parameters were observed over the time in HIV mono-infected patients. This finding is comparable with studies from Ethiopia **(**Yitayih et al., 2013). However, there was significant elevation of AST, ALT, ALP, conjugated bilirubin and total bilirubin in HIV/HBV co-infected patients over the time. Liver enzymes AST, ALT ALP levels have been reported by Obi et al (Simon et al., 2014) to be significantly higher in co-infection with hepatotropic viruses compared with mono-infection and control group under antiretroviral therapy (Otegbayo et al., 2008) in south western Nigeria had similar result; (Ballah et al., 2012) in the same environment and (Ibeh et al., 2013) in Eastern part of Nigeria. This elevation of biochemical parameters could be due to hepatocellular damage, jaundice, cytolysis or cholestasis.

CONCLUSION

ALT was significantly higher among HIV/HBV co-infected participants compared to those with HIV alone in first blood sample. The second blood sample after 3 months in HIV mono-infected patients presented no significant elevation of livers biomarkers. However, in HIV/HBV co-infected patients, the results has shown significant elevation of AST, ALT, ALP, conjugated bilirubin and total bilirubin, while the ã-GT remained stable over time.

ABBREVIATION'S LIST

ALP: alkaline phosphatase
ALT: alanine amino transaminase
AST: aspartate amino transaminase
CB: conjugated bilirubin
CD4: cluster of differentiation 4
HBV: hepatitis B virus
HBsAg: Hepatitis B surface Antigen
HIV: human immunodeficiency virus
g-GT: Gamma Glutamyl transaminase
TB: total bilirubin

ACKNOWLEDGEMENTS

We would like to thank the Yaoundé central hospital/ Day care unit. Our special thanks and appreciation also goes to all the study participants who voluntarily participated in this study. Last but not the least; we also thank Modernly Reference laboratory personnel for their consistent support with some reagents and other materials during the project. Our special thanks goes to Dongmo Lekagne Blaise for his contribution in statically analysis.

REFERENCES

Ballah AB, Ajayi B, Abja AU, Bukar AA, Akawu C, Ekong E. (2012). A survey of hepatitis B and C virus prevalence in HIV positive patients in a tertiary health institution in North Eastern Nigeria. International journal of Medicine and Medical Science. 4, 13-18.

Dieterich DT. (2007). Special considerations and treatment of patients with HBV-HIV co-infection. Antivir Therapy. 12, H43-H51.

Diop-Ndiaye H, Toure-Kane C, Etard JF, LO G, Diaw P., Ngom-G NF. (2008). Hepatitis B, C seroprevalence and delta viruses in HIV-1 Senegalese patients at HAART initiation (retrospective study) Journal of Medical Virology. 80, 1332–1336.

Gupta S, Singh S. (2010). Occult hepatitis B virus infection in ART-naive HIV-infected patients seen at a tertiary care centre in north India. BMC Infectious Disease. 10, 53-58.

Ibeh BO, Olu O, Modamiro OD, Ibeh U, Habu JB. (2013). Biochemical and Haematological changes in HIV subjects receiving Winnie cure

antiretroviral drug in Nigeria. Journal of Biomedical Science. DOI: 10.1186/1423-0127-20-73.

Laurent C, Bourgeois A, Mpoudi-N E, Kouanfack C, Ciaffi, Nkoue N. (2010). High rates of active hepatitis B and C co-infections in HIV-1 infected Cameroonian adults initiating antiretroviral therapy. Pub Med 11, 85–89.

Nyirenda M, Beadsworth M, Stephany P, Hart C, Hart I, Munthali C. (2008). **Prevalence of infection with hepatitis B and C virus and co-infection with HIV in medical inpatients in Malawi.** *Journal of Infectious Disease.* **57,** *72–77.*

Ochola E, Ocama P, Orach CG. (2013). High burden of hepatitis B infection in Northern Uganda: Results of a population-based survey. BMC Public Health. DOI: 10.1186/1471-2458-13-727.

Odenyo H, Schoub B, Ally R, Kairu S, Segal I. (2000). **Hepatitis B and C virus infections and liver function in AIDS patients at Chrishanibaragwanath hospital Johannesburg.** *East African Medical Journal.* **77,** *13–15.*

Olawumi HO, Olanrewaju DO, Shittu AO, Durotoye IA, Akande, Nyamngee A. (2014). Effect of Hepatitis-B Virus Co-Infection on CD4 Cell Count and Liver Function of HIV Infected Patients. Ghana Medicine Journal. 48, 96–100.

Olufemi A, Emmanuel A, Zaccheus A, Ibrahim W, Funmilayo E, Patience A. (2009). **Hepatitis B and C virus co-infection in Nigerian patients with HIV infection.** *Journal of Infectious Dev Ctries.* **3**, *369–375.*

OMS: Organisation mondiale de la santé. Rapport mondial de l'hépatite B. (2015).

Otegbayo JAL, Taiwo BO, Akingbola TS, Odaibo GN, Adedapo KS, Penugonda S, Adewole IF, Olaleye DO, Murphy R, Kanki P. (2008). Prevalence of hepatitis B and C seropositivity in a Nigerian cohort of HIV-infected patients. Ann Hepatol. 7, 152-156.

Pratt DS, Kaplan MM. (2000). Evaluation of abnormal liver enzyme results in asymptomatic patients. England Journal of Medicine. 342, 1266–711.

Saravanan S, Velu V, Kumarasamy N, Nandakumar S, Murugavel KG, Balakrishnan P. (2007). Co-infection of hepatitis B and hepatitis C virus in HIV-infected patients in south India. World Journal of Gastro Enterology .13, 5015-5020.

Simon OO, Haruna A, Baba, Marycelin M, Grace I. Amilo, Alhaji B. (2014). The Effect of Co-infection of HIV and Hepatotropic Viruses on Selected Biochemical and Haematological Markers of Patients in Northeastern Nigeria. International Journal of Tropical Disease & Health 4, 568-58

Swati G, Sarman S. (2006). Hepatitis B and C virus co-infections in human immunodeficiency Virus positive North Indian patients. World Journal of Gastroenterology. 12, 6789-6883.

Vincent S, Massimo P, Maurizio B. (2005). Care of patients with chronic hepatitis B and HIV co-infection: recommendations from an HIV-HBV international panel. 19, 221-240.

WHO global report on HIV/AIDS (2015).

Yitayih W, Meseret A, Fanaye A, Yeshambel B. (2013). HBV and HCV seroprevalence and their correlation with CD4 cells and liver enzymes among HIV positive individuals at university of

Gondar teaching hospital, northwest Ethiopia. Virology Journal **10**, 2-8.

Zhou J, Dore GJ, Zhang F, Lim PL, Chen YM. (2007). HIV Observational Database. Hepatitis B and C virus co-infection in The TREAT Asia HIV Observational Database. Journal of Gastro Enterology. 22, 1510-1518.

22. Zoufaly A, Onyoh E.F, Tih PM, Awasom CN, Feldt T. (2012). High prevalence of hepatitis B and syphilis co-infections among HIV patients initiating antiretroviral therapy in the north-west region of Cameroon. International Journal of STD AIDS. 23, 435–438.

Received: 31/09/16
Accepted: 10/11/16