Research Article

IMPORTANCE OF BIOCHEMICAL EXPLORATION OF THE LIVER IN THE CONTROL OF DISEASE PROGRESSION IN PEOPLE LIVING WITH HIV/AIDS AND CO-INFECTED BY HIV AND HEPATITIS C VIRUS IN CAMEROON.

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ABSTRACT

Viral infection continues to be a major cause of morbidity and mortality in the world in particular those caused by the Hepatitis C virus (HCV) and the Human Immunodeficiency virus (HIV). The aim of this study was to evaluate the importance of the biochemical exploration of the liver function in the control of disease progression in people living with HIV/AIDS and co-infected by HIV and hepatitis C virus. Sera from 75 patients positive for HIV were screened for HCV antibody by immuno-chromatographic test in Yaoundé central hospital, from November 2015 to February 2016. The biochemical parameters (AST, ALT, CB, TB, γ-GT, ALP and creatinin), as well as CD4 T cell level determination were assessed following standard procedures. A second blood sample was taken from HIV mono-infected and HIV/HCV co-infected after three months. Socio-demographic data were also collected. The data were entered and analyzed using SPSS version 22.1 statistical software and $p<0.05$ was considered as statistically significant.

Amongst 75 study patients, 10 (13.3%) were HIV/HCV co-infected. The results obtained showed that, the activity of γ-GT was significantly higher ($P <0.0001$) in HIV/HCV co-infected patients compared to HIV mono-infected patients. The concentration of total bilirubin was also significantly higher in HIV/HCV co-infected patients ($P = 0.015$). Biochemical analysis using the second blood sample carried out 3 months later, after first sample, showed a significant increase of creatinin observed in HIV mono-infected patients, but no significant increase of some biochemical parameters (ALT, Creatinin, total Bilirubin, conjugated Bilirubin) was observed in HIV/HCV co-infected patients.

In HIV / HCV co-infected patients, the first blood sample showed a significant increase of γ-GT (118.36±75.95 IU) compared to HIV mono-infected patients, in the Yaoundé Central Hospital. From this observation, hepatic damage should be evaluated by analyzing biochemical parameters.

Key words: Liver enzyme, Co-infection, HIV, HCV, control

RESUME

Les infections virales continuent toujours d'être une cause majeure de mortalité et de morbidité dans le monde en particulier celle causée par le virus de l'hépatite C (VHC). Le but de cette étude était d'évaluer l'importance de l'exploration biochimique 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 C au Cameroun. Les sérums de 75 patients positifs au VIH ont été prélevés pour la détermination de l'anticorps du Virus de l'hépatite C (Ac-VHC) par le test immuno-chromatographique à l'Hôpital Central de Yaoundé, de Novembre 2015 à Février 2016. Les paramètres biochimiques (ASAT, ALAT, BC, BT,γ-GT, PAL et la créatinine), 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/VHC 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.

Sur les 75 patients, 10 (13,3%) étaient co-infectés par le VIH/VHC. Les résultats obtenus avaient montré que, l'activité de la γ-GT était significativement élevée chez les patients co-infectées par le VIH/VHC (P <0,0001) comparés aux patients mono-infectés VIH. La concentration de la bilirubine totale était aussi significativement élevée chez les patients co-infectés VIH/VHC (P = 0,015). Les analyses biochimiques du second prélèvement effectuées après trois mois du premier prélèvement ont montré une élévation significative de la concentration de la créatinine chez les patients mono-infectés par le VIH, mais aucune élévation significative des paramètres (ALAT, Créat, bilirubine totale, bilirubine conjuguée) n'avait été observé chez les patients co-infectés par le VIH/VHC.

Le premier prélèvement a montré une élévation significative de la γ-GT (118,36±75,95 IU) chez les patients co-infectés comparé aux mono-infectés VIH à l'Hôpital Central de Yaoundé. A partir de cela, les dommages hépatiques doivent être évalués par l'analyse des paramètres biochimiques.

Mots clés: enzymes hépatiques, Co-infection, VIH, VHC, contrôle

INTRODUCTION

Diseases of the liver are a major problem in patients living with the Human Immunodeficiency Virus (HIV). Liver inflammation caused by hepatitis C virus is a major public health problem in the world in general and particularly in Africa and Asia (East and Central) (OMS, 2015). There is an estimated 130 to 150 million people with viral hepatitis C worldwide (OMS, 2015) including 500 000 deaths each year (LOZANO, 2010). In Cameroon, the prevalence of HCV was found to be 1.03 %, a prevalence that increased significantly with age (EDS-MICS, 2011; NJOUOM and TEJIOKEM, 2016) and for HIV, the prevalence was 4.3 % among adults 15 to 49 years (EDS-MICS, 2011). The hepatitis B and C share with the Human Immunodeficiency Virus (HIV) certain transmission routes, thus favouring the possibility of co-infection (ZEBA, 2012). These similar routes of contamination, infection by HIV is often associated with HCV (VOGEL et al., 2009). However, studies reported by SHARIFI and METANAT, 2006 show that the rates of co-infection of HIV with either HCV vary from region to region, study population and risk factors for HIV acquisition. In HIV/HCV co-infections, there is a more rapid progress to cirrhosis, end-stage liver disease and hepatocellular carcinoma (ROMEO et al., 2000). The impact of HCV is not limited in causing liver hepatotoxicity but also results in failure of immunological recovery in HIV positive patients. While, co-infection with HIV alters the natural history of HBV disease, the direct impact of HCV upon HIV disease progression remains controversial in many reports (SULKOWSKI, 2008). A raised serum liver enzyme level such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) reflects direct hepatocellular damage or liver dysfunction. In fact, the activities of ALT and AST have been reported in Nigeria (SIMON et al., 2014; BALLAH et al., 2012) to be significantly higher in sera of patients co-infected with hepatotoxic viruses compared with mono-infection and control group under antiretroviral therapy. Although biochemical parameters could serve as pointers for early detection of liver disease in HIV patients, to our knowledge, no published work has presented the changes of biochemical parameters in patients co- infected with HIV / HCV in Cameroon. In order to ensure a better monitoring of treatment, we conducted this study with the objective to evaluate the importance of the biochemical exploration of the liver function in the follow up of disease progression in people living with HIV/AIDS and co-infected by HIV and hepatitis C virus in Yaoundé Central Hospital, Cameroon.

METERIALS AND METHODS

Materials

Biological material (serum, plasma) and lab equipment were used in this study.

Study design, area, and period

This was a prospective and analytical study from November 2015 to February 2016, at the Yaoundé Central Hospital.

Source population and study participants

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.

Inclusion and exclusion criteria

Participants in this study were aged over 21 years irrespective of gender, ethnicity, religion and tribe. Enrolled participants were patients who agreed to sign a consent form after being informed of the nature of the study, the potential benefits and foreseeable risks as well as the voluntary nature

of the participation. 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.

Data 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 HCV 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 the serum used for determination of liver enzyme levels within one hour of separation. The remaining serum kept in deep refrigerator ("20°C) until detection of HCV.

Statistical analysis

Data were subjected to statistical analysis for medium and significant differences using SPSS 22.1 and Excel 2010 (graphics). ANOVA (Analysis of variance) 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

This study received an ethical clearance from the Cameroon National Research Ethics Committee for Human Health. N° 2015/11 /664/CNERSH /SP. In addition, informed consent of participants was obtained prior to their enrollment.

EC numbers of enzymes

ALP (alkaline phosphatase) EC: 3.1.3.1

ALT (alanine amino transaminase) EC: 2.6.1.2

AST (aspartate amino transaminase) EC: 2.6.1.1

γ-GT (Gamma Glutamyl transferase) EC: 2.3.2.2

RESULTS

Socio-demographic characteristics

In the present study, serum of 75 HIV positive patients was tested for hepatitis C Antibody-HCV (HCV-ab). A total of 10 patients were found to be positive for Ac-HCV i.e the prevalence rate was found to be 13.3%. Out of these 10 patients, 3 (4%) were male and 7 were female 7(9.3 %). The average age was 54.48 ± 7.58 years.

Comparison between HIV mono-infected and HIV/HCV co-infected groups.

In HIV mono-infected study participants, the mean serum AST, ALT, γ-GT , ALP, TB, CB and creatinin were 33.41±21.38 International Unit (IU), 21.17±8.07 IU , 39.40±33.20 IU, 103.38±37.26 IU, 0.22±0.32 mg/dL, 0.020±0.019 mg/dL and 0.92±0.31 mg/dL respectively. In HIV/HCV co-infected study participants, only the increase of γ-GT activity (118.36±75.95IU) and TB (1.36±3.80 IU) were obtained. The mean CD4 count in HIV mono-infected patients was 413.68±192.49 cells/mm^3 and the mean CD4 count in HIV/HCV co-infected was 369.90±235.03 cells/mm^3 respectively. However, the difference was not statistically significant (**table 1**).

Type of infection	HIV mono-infected	HIV/HCV co-infected	P value
Size	65 (86.7%)	10 (13.3%)	$X^2 = 40.33$ ($P < 0.0001$*)
AST (IU)	33.41±21.38	42.44±23.00	P = 0.288
ALT (IU)	21.17±8.07	24.78±15.98	P = 0.271
γ-GT (IU)	39.40±33.20	118.36±75.95	P < 0.0001*
ALP(IU)	103.38±37.26	91.74±40.89	P = 0.390
Creat (mg /dL)	0.92±0.31	1.05±0.20	P = 0.190
TB (mg/dL)	0.22±0.32	1.36±3.80	P = 0.015*
CB (mg/dL)	0.020±0.019	0.055±0.11	P = 0.054
Rate of CD4(cells/mm^3)	413.68±192.49	369.90±235.03	P = 0.515

Mean of biochemical parameters and their association with HIV mono-infection and HIV/ HCV co-infection in the second blood sample

After three months, the results showed significant elevation of creatinin in HIV mono-infected groups and, no significant elevation in HIV / HCV co-infected patients **(table 2**).

Table 2: means of some parameters in HIV/HCV co-infected and HIV mono-infected groups at recruitment and after 3 months

HIV-HCV	Mean serum ALT (IU)	Mean serum AST (IU)	Mean serum creat (mg/dL)	Mean conjugated bilirubin (mg/dL)	Mean total bili (mg/dL)
At recruitment	22.17± 15.53	37.21± 27.13	1.06± 0.16	0.027±0.009	0.154± 0.074
After 3 months	35.07±15.90	31.13± 22.76	1.51±0.25*	0.032± 0.025	0.218± 0.041
HIV ALONE	Mean serum ALT (IU)	Mean serum AST (IU)	Mean serum creat (mg/dL)	Mean conjugated bilirubin (mg/dL)	Mean total bili (mg/dL)
At recruitment	20.47± 7.17	30.08± 12.57	0.87± 0.30	0.021±0.017	0.26±0.41
After 3 months	16.97± 18.41	27.84± 25.16	1.40±0.49	0.029±0.043	0.23±0.44

DISCUSSION

In this study, HIV/HCV co-infection rate was 13.3% which is almost equal to study done in South Africa (13.4%), in Nigeria (14.7%), (PARBOOSING et al, 2008; TAIWO et al., 2012). But it is more or less comparable with, 5% (YITAYIH et al., 2013), 10.6% (NTAGIRABIRI et al., 2012**)**. Generally, as several studies reported and anticipated in different parts of the world, such co-infection differences could be due to differences in geographic regions, types of risk groups and the means of exposures involved (LESI et al., 2007). In this study, there was no significant difference in CD4 T cell count between HIV mono-infected (413.68±192.49 cells/mm^3) and HIV/HCV co-infected (369.90 ±235.03 cells/mm^3) patients. This observation was also made in Ethiopia (YITAYIH et al., 2013). This result is comparable with a mean CD4 T cell count of 260 cells/mm^3, 274 cells/mm^3 and 288.6 cells/mm^3 that were reported in studies conducted in Nigeria, Ethiopia and India respectively (OLUFEMI et al., 2009; YITAYIH et al., 2013; TRIPATHI et al., 2007). However, HIV-HCV co-infected patients had relatively lower mean CD4 T cell values than HIV mono-infected patients.

The activity of γ-GT and the concentration of total bilirubin were significantly higher in HIV/ HCV co-infected patients compared to HIV mono-infected patients. This result could be attributed to the fact that hepatitis C virus is a hepatotrophic virus that elicits an immune response that causes liver damage which was revealed.

Liver enzymes such as AST, ALT have been reported by SIMON et al., (SIMON et al., 2014) to be significantly higher in co-infection with hepatitis C virus compared with HIV mono-infection and control group under antiretroviral therapy. Ballah et al., (BALLAH et al., 2012) in North Eastern Nigeria had similar results. Another report in Nigeria (FOLARANMI, 2014) showed a significantly higher mean plasma value of ALT in HIV co-infected patients with hepatitis C virus compared with the HIV mono-infected patients with a $p<0.01$ and no significant difference in the mean plasma value AST and Total Bile Acids in HIV patients co-infected with hepatitis C virus compared with the HIV mono-infected patients with a $P> 0.01$. However, in a study which was conducted in Ethiopia (YITAYIH et al., 2013), despite the absence of statistically significant difference in the mean levels of the liver enzymes

between HIV mono-infected and HIV/HCV co-infected patients, raised ALT, AST and ALP were found in HIV/HCV co-infected patients. Otherwise, significantly raised ALT was observed in 20 % of HIV/HCV co-infected patients in India (TRIPATHI et al., 2007). The elevation of biochemical parameters could be due to a hepatocellular damage, jaundice, cytolysis or cholestasis. Liver transaminases are useful biomarkers of liver injury in individuals with some degree of intact liver function. The variation of these liver enzymes may be also due to the duration of the viral hepatitis infection as well as the patient's condition like having chronic alcoholism or other drug induced hepatotoxicity that may contribute to the development of liver fibrosis and raised liver enzyme levels. Indeed, HCV infection is aggravated in case of HIV infection. This justifies the fact that, HCV exerts significant pressure on the liver and liver cells that can lead to the destruction of liver cells by the immune system. In the case of viral hepatitis (HCV), lysis of hepatocytes is not the result of a viral cytopathic effect, but the action of cytotoxic lymphocytes in infected hepatocytes. Furthermore, HCV causes a localized inflammatory reaction in the liver which allows the virus to infect and progressively destroy liver tissues. Liver failure occurs only when most of the liver cells are destroyed.

The elevated creatinin observe after three months in HIV mono-infected can be result of antiretroviral toxicity and the influence that virus would have on the kidneys may cause undesired operation. The non-significant elevation of biochemical parameters (ALT, PAL, creatinin, total Bilirubin and conjuged bilirubin) in co-infected patients can be due to the constant pressure exerted by the two viruses on the liver and kidneys, which could cause either cytolytic or malfunction of the gallbladder or hemolysis. This could also result from antiretroviral toxicity because these patients are already under treatment during these three months.

CONCLUSION

In HIV/HCV co-infected patients, γ-GT, total bilirubin were significantly higher compared to HIV mono-infected patients in the first sample. After 3 months, no significant increase of biochemical parameters was observed in HIV/HCV co-infected patients. HIV/HCV co-infection damages the liver more than single HIV infections.

ABBREVIATION' S LIST

ALP: alkaline phosphatase
ALT: alanine amino transaminase
AST: aspartate amino transaminase
CB: conjugated bilirubin
Creat: creatinin
CD4: cluster of differentiation 4
ELISA: Enzyme Linked Immunosorbent Assay
HCV: hepatitis C virus
HCV-ab: Hepatitis C virus antibody
HIV: human immunodeficiency virus
γ-GT: Gamma Glutamyl transferase
SD: Standard deviation
TB: total bilirubin

COMPETING INTERESTS

The authors declare that they have no competing interests.

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