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SELENIUM AS ADJUNCT TO HAART IN THE MANAGEMENT OF HIV/HEPATITIS B VIRUS CO-INFECTION: A RANDOMIZED OPEN LABEL STUDY

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ABSTRACT

Objective: Management of viral hepatitis in patients with HIV disease is quite challenging and complex. With effective HIV treatment, people with HIV/HBV co-infection are living longer. HBV epidemiologic surveys showed an inverse association between selenium (Se) level and regional cancer incidence, as well as viral infection. This study assessed effect of selenium as adjunct to HAART in management of HIV/HBV co-infection.

Methods: A randomized open label study with participants allocated into three treatment groups: - HAART-only, Se-only and HAART-plus-Se. HIV viral load, HBV viral load, CD4 cell count, and *alanine aminotransaminase* (ALT), were analyzed at baseline and 18th month, analyzed using SPSS 5 v11. Ethical approval was obtained from Institute's Ethical Review Board.

RESULTS: Sample size was 149 HIV/HBV patients. Rate of HBV clearance was higher among those on HAART-plus-Se at 18th month when compared with rate of clearance among those on HAART-only (p=0.046). CD4 count increment among HAART-plus-Se group compared with HAART-only group was higher (p=0.133), though not statistically significant. On comparison of baseline and 18th month ALT, there was significant decline for HAART-plus-Se (p=0.002) compared with HAART-only group.

CONCLUSION: Selenium seems to have protective effect on liver cells; may be beneficial as adjunct to HAART in HIV/HBV management.

Key words: HIV/HBV co-infection, Selenium adjunct, HAART

SELENIUM COMME COMPLEMENT A HAART DANS LA GESTION DE LA CO-INFECTION DU VIH/VIRUS DEL'HEPATITE B : UNE ETUDE OUVERTE RANDOMISEE.

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RESUME

Objectif: La gestion de l'hépatite virale chez les patients atteints du VIH est assez difficile et complexe. Avec un traitement efficace contre le VIH, les personnes atteintes la co-infection du VIH/VHB vivent plus long. Enquêtes épidémiologiques du VHB ont montré une association inverse entre le niveau du sélénium (Se) et l'incidence du cancer régionale, ainsi que l'infection virale. Cette étude a évalué l'effet du sélénium comme complément à HAART dans la gestion de la co-infection du VIH/VHB.

Méthodes : Une étude ouverte randomisée avec les participants alloués en trois groupes de traitement : HAART – seulement, Se-seulement et HAART – plus- Se. La charge virale du VIH, la charge virale du VHB, la numération des CD4 et alanine aminotransaminas (ALT), ont été analysés au base et au 18^{ème} mois, analysés en utilisant SPSS 5 v11. L'approbation éthique a été obtenue au comité d'éthique d'examen de l'institut.

Résultats : Le numéro de l'échantillon était 149 patients de VIH/VHB. Le taux de clairance du VHB était plus élevé chez les personnes sur HAART-plus-Se au 18^{ème} mois par rapport à la vitesse de clairance parmi ceux sur HAART – seulement (p=0,046). L'incrément de CD4 parmi le groupe HAART – plus- Se comparé au groupe HAART – seulement était plus élevé (p=0,133), quoique pas statistiquement significative. Sur la comparaison base et ALT de 18^{ème} mois, il y a eu baisse significative pour HAART – plus – Se (p=0,002) par rapport au groupe de HAART – seulement.

CONCLUSION: Le sélénium semble d'avoir un effet protecteur sur les cellules du foie; peut être bénéfique comme complément à HAART dans la gestion du VIH/ HBV.

Mots clés : La co-infection VIH/ HBV, complément de Sélénium, HAART.

INTRODUCTION

About 350 million persons are chronically infected with hepatitis B virus (HBV) and are at high risk of death from active hepatitis, cirrhosis and primary hepatocellular carcinoma [1,2]. The rate of liver-related death is higher in HIV/HBV co-infected persons compared with those who have HIV infection alone [3]. With effective HIV treatment, people with HIV/HBV co-infection are living longer lives. Slower-progressing liver disease caused by hepatitis B, such as liver cancer and liver failure, are now becoming key health concerns. End-stage liver disease (ESLD) from viral hepatitis co-infection is now a leading cause of death among people with HIV [4]. The available reports show that, it is currently not possible to completely cure HBV with treatment, since small particles of the virus (cccDNA) inserts itself inside the nucleus of liver cells, where the drugs cannot penetrate.

However, drugs can control HBV in order to prevent or delay development of liver damage. The reported burden of hepatitis B infection may be a tip of the iceberg as occult infection is seen more commonly in HIV-positive individuals and often go undiagnosed. This is especially so in low income countries with poor diagnostic infrastructure.

Clinically the coexistence of the two infections leads to an increased risk of hepatic cirrhosis and hepatocellular carcinoma if not treated [5]. Early identification of the coexistence of the two infections and prompt treatment is therefore essential in not only reducing the associated mortality but improved quality of life.

While the use of highly active antiretroviral therapy (HAART) has been shown to change the natural course of HIV and effective treatment available for persons with HBV, the management of HIV/HBV co-infection is quite challenging. This is especially so when pharmacologic therapy is used, making it difficult to identify the ideal strategy for management of the co-infection. Selenium (Se) has been reported to play a crucial role in the detoxification of liver enzymes, enhancing immune function as well as providing antioxidant effect [6]. Yu, *et al* [7] reported that epidemiological surveys demonstrated an inverse association between Se level and regional cancer incidence, as well as HBV infection. He further reported that a four-year animal study showed that dietary supplement of Se reduced the HBV infection by 77.2% and liver precancerous lesion by 75.8%.[8] Qia *et al* reported beneficial effects of Se, vitamin E, and beta-carotene on cancer mortality [9]. It has been reported that Se appears to be protective in individuals infected with hepatitis virus (B or C), against the progression of the condition to liver

cancer [10]. Rayman, in the review of the evidence and mechanism of action of Se in cancer prevention, reported evidence associating Se ingestion with reduction of DNA damage and oxidative stress [10]. The data showing an effect of selenoprotein genotype on cancer risk implied that selenoproteins were indeed implicated. Khan *et al* [11] recently reported that serum Se concentration of hepatitis B and C patients is less than serum Se concentration of healthy individuals and that, serum Se decline is relative to increased severity of disease [12].

This study was conducted to evaluate the effect of selenium as adjunct to HAART in the management of patients co-infected with HIV/HBV co-infected patients. Selenium was found to be reasonably beneficial in addition to HAART in the management of this group of patients.

MATERIALS AND METHODS

Ethics statement: Ethical approval was obtained on application from the Institute's (NIMR) Ethical Review Board.

Study setting: The study was conducted at the HIV treatment centre, Nigerian Institute of Medical Research (NIMR), in Lagos. NIMR is the apex medical research institution in Nigeria charged with the responsibility to conduct research into disease of public health importance in the country. At the initiation of the Federal Government of Nigeria antiretroviral drug access programme in 2002, it was selected as one of the 25 treatment centres primarily to provide the research backup for the National HIV programme. Patients were enrolled into the HIV treatment programme on referral from the HIV Counselling and Testing Centre (HCT), NIMR Lagos or transferred from other government HIV treatment centres. Presently the centre provides free comprehensive HIV care, treatment and support for over 19,000 patients.

Study Design: A randomized open label study with participants randomly allocated into three treatment groups: HAART only, Selenium only and HAART+Selenium. The target population was, HIV-1 sero-positive adult males and females, confirmed by Western Blot or a licensed double ELISA procedure, antiretroviral drug naïve, and who were HBsAg positive and whose baseline serum ALT (*alanine transaminase*), CD4⁺ lymphocyte counts have been evaluated and viral loads quantified by standard method at the Human Virology Lab in NIMR. The detection limit for the HIV viral load assay was 400 RNA copies/mL (standard method) and 316 DNA copies/mL for HBV.

Sample size calculations: The sample size calculation was based on two-proportion method from Epi-calculator in *Epi Info v6*. One hundred and forty-nine (149) consenting HIV/HBV patients aged 18 years and above were counselled and enrolled for the study between 2006 and 2008 (figure 1). Excluded from the study were participants who declined consent and/or are on previous or ongoing treatment with anti-retroviral agents. A flow chart of patient progress through the study is shown in figure 2.

Treatment Groups: Group 1 (HIV/HBV only) on Truvada [Tenofovir (TDF) 300mg plus Emtricitabine (FTC) 200mg] with tablet Nevirapine (NVP) 200mg once daily for 14 days, then 12 hourly in the absence of severe adverse drug reaction, or Efavirenz (EFV) where NVP is not applicable.

Group 2 (HIV/HBV + Selenium): HAART regimen as in Group 1 plus 200 mcg selenium daily.
Group 3 (Selenium only): HAART ineligible patients on 200 mcg selenium daily.

Laboratory tests

CD4 Lymphocyte cell count: CD4 counts were determined for each patient at baseline and subsequently on 3 monthly intervals, using the *Dynalbead* Technique (Dynal A.S. Oslo, Norway). This was the adopted technique for CD4 estimation under the national ARV programme at that time. The CD4 was expressed as cells/ μ l of blood.

Viral load estimations (HIV-1 RNA and HBV DNA): The viral load of each patient was quantified with 200 μ l plasma samples using the *Amplicor HIV-1 monitor* version 1.5 (Roche Diagnostic Systems, Branchburg, NJ, USA) and expressed as RNA copies/ml. The non-detectable limit of the standard method used in this study was 400 copies/ml. Hepatitis B markers determined were HBsAg and HBV DNA viral load. HBV DNA was measured quantitatively using the *COBAS AMPLICOR* technique by Roche (Germany). The minimum detection level of the *COBAS AMPLICOR* was 316 DNA copies/ml. Therefore undetected was recorded as equal to or less than 316 DNA copies/ml.

Alanine transaminase (ALT): Commercially available ELISA kits (*RANDOX*) were used to assay for the serum ALT.

Follow up: The patients were monitored for their response to antiretroviral therapy, through an initial two 3-monthly evaluation of all parameters estimated at baseline, (except HBV viral load, which was repeated only at 18th month) as treatment progressed and thereafter 6 monthly. Data Management: Data for the study was collected using a case record form

designed for the study. Obtained data was entered and analysed using SPSS 5 v11. Comparisons were made between groups using student t-test. Summary descriptive statistics was carried out on the demographic characteristics; baseline, 18th month data, ALT, CD4 count, HBV and HIV viral loads, were compared between the treatment groups and p-value less than 0.5 was taken as significant.

RESULTS

Age and sex distributions, of HIV/HBV population showed female (59.9%) preponderance. The preponderant age groups were 26-35 (41.5%) and 36-45 (36.1%) years. Twenty one (21) participants were lost to follow up. Seven were confirmed dead within the study period, (all the seven were HIV/HBV/TB co-infected). Only 121 who completed the study had repeated HBV DNA analysis at 18th month. At baseline, 32.9% of all the participants had undetectable HBV viral load, and at the end of the study 72.2% had undetectable HBV viral load. There was a significant decrease in HBV viral load of all patients who were on HAART plus Se at 18th month when compared with baseline data ($p=0.001$). All participants (100%) who had undetectable HBV viral load at baseline, were eligible and initiated HAART, remained HBV undetected at the end of the study. While only 68% of those who were not on HAART, and had undetectable HBV viral load at baseline, remained undetectable at 18th month (Table 1).

The rate of HBV clearance was higher among those on HAART+Se, when compared with the HAART-only group ($p=0.046$). The CD4 cell count increment among HAART+Se group compared with HAART-only group was higher ($p=0.133$), though not significant. On comparison of baseline and 18th month ALT, there was a significant decline for HAART+Se treatment group ($p=0.05$) compared with HAART-only group ($p=0.257$).

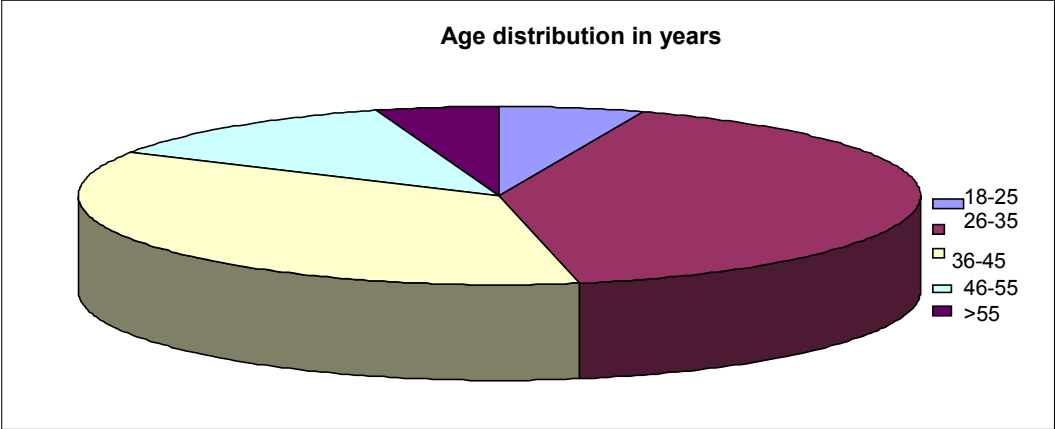


FIGURE 1: AGE DISTRIBUTION OF STUDY PARTICIPANTS

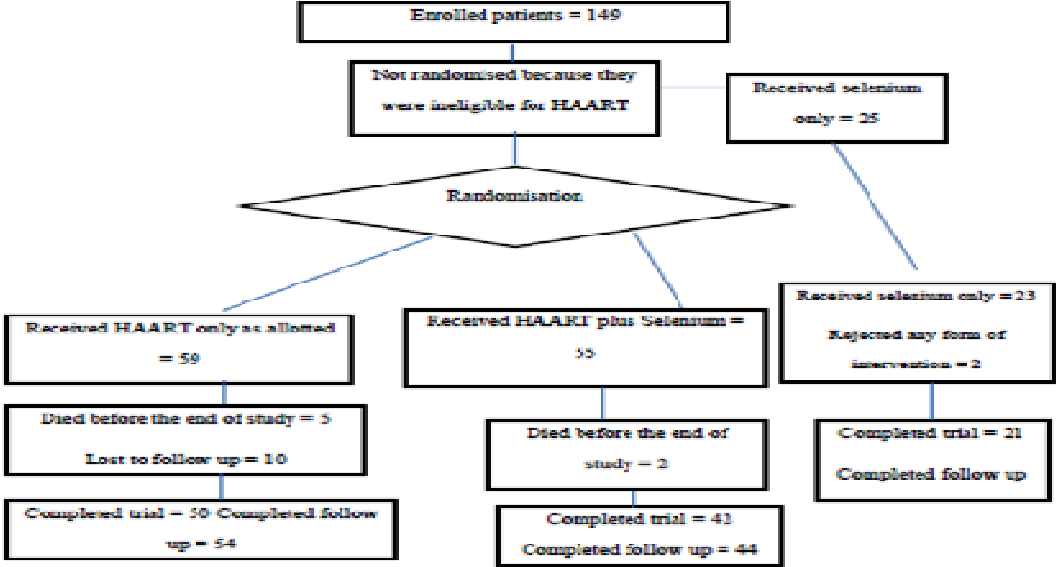


FIGURE 2: FLOW CHART DESCRIBING PROGRESS OF PATIENTS THROUGH STUDY

TABLE 1: COMPARISONS BETWEEN BASELINE AND 18TH MONTH DATA FOR ALL THE TREATMENT GROUPS

Parameters measured	TG 3 (N = 23) Se only			TG 2 (N = 42) HAART +Se			TG 1 (N =50) HAART only		
HBV VL (DNA copies/ml)	Baseline	18 th month	P value	Baseline	18 th month	P value	Baseline	18 th month	P value
	2641	316	0.019	303,502	316	0.001	86,790	316	0.012
Median	316-2109260 316-1961980			316-6469800 316-10362			316-6259400 316-1604300		
Range									
HIV VL (RNA copies /ml)	Baseline	18 th month	P value	Baseline	18 th month	P value	Baseline	18 th month	P value
	8773	3066	0.013	155638	200	0.001	54,720	200	0.003
Median	200-304648 200-149550			200-487443 200-76861			200-2692625 200-26735		
Range									
CD4 Count (cell/μl)	Baseline value	18 th month	P	Baseline	18 th month	P value	Baseline	18 th month	P value
	389	484	0.435	103	344	0.001	143	309	0.01
Median	218-889 212-942			8-616 66-999			13-560 42-780		
Range									
ALT (IU/ml)	Baseline value	18 th month	P	Baseline value	18 th month	P	Baseline value	18 th month	P
	27	22	0.440	34	21	0.05	26	22	0.257
Median	11-65 12-50			5-327 8-58			11-208 7-192		
Range									

Key: ND HBV= undetectable HBV viral load; ND HIV = undetectable HIV viral load, TG 3 = treatment group 3; TG 2 = treatment group 2; TG 1= treatment group 1; Statistically significant p value ≤0.05

TABLE 2: NOT DETECTED HBV AND HIV VIRAL LOADS FREQUENCIES AFTER 18 MONTHS FOR THE STUDY GROUPS

Parameters measured	TG 1 (N =50) HAART only	TG 2 (N = 42) HAART +Se	TG 3 (N = 23) Se only
% ND HBV at baseline	27.3%	31.6%	28.6%
% ND HBV at 18 th month	67.3%	83.8%	25%
% ND HIV at baseline	7.3%	5.3%	28.6%
% ND HIV at 18 th month	81.8%	81.6%	14.2%

DISCUSSION

At baseline, 27.3% and 7.3% of the participants on HAART only had undetectable HBV and HIV viral load respectively, and at the end of the 18th month, 67.3% and 81.8% had undetectable HBV and HIV viral loads respectively.

There was significant difference between baseline and 18th month HBV and HIV viral loads for this group. The HBV and HIV viral loads significantly reduced over the 18-month period as anticipated with use of ARVs suitable for HIV/HBV co-infection. ALT demonstrated mild changes during the study period while CD4 increased, though the difference was not

statistically significant. However, it may indicate some recovery of immune system with institution of HAART in this group. The HBV and HIV viral loads decreased significantly over the duration of study. This is somewhat similar to what was seen with the HAART-only group; however the CD4 increase was comparatively higher, probably indicating the antioxidant support provided by the co-administration of selenium. There was also a noticeable improvement on the ALT activity in this treatment group. This may indicate that selenium is likely to have a protective effect on the hepatic cells and probably normalizes the liver enzymes. This supports some of the earlier reports on selenium [13] of

beneficial effect of selenium on symptomatic hepatitis C patients with elevated aminotransferases when placed on a triple antioxidant therapy comprising alpha lipoic acid, selenium and 'Silybummarianum'(milk thistle). All the patients were reported to have been spared hepatic transplantation, for they had improved laboratory indices, and returned to normal working life. [14] Schwarz et al described the relationship between selenium intake by food and prevention of liver necrosis in rats. All the parameters were slightly improved over the 18-month period, which could be a pointer that sole administration of Se compared to ART has limited effect on the HBV and HIV replication.

It was observed that there was a relative stability and improvement of HBV viral load but deterioration of the HIV viral load among the selenium only group. It may be adduced that there is need for intervention for every HIV/HBV infected patient. Six patients opted out of any intervention measure (Se or HAART). This group though, a deviation from the original study design was followed up along with the intervention groups. It was observed that their HBV and HIV viral loads were increased after 18 months while the CD4 cell counts decreased significantly. This observation probably depicts the natural history of HIV/HBV co-infection in our setting. The changes noticed over the period of observation were probably facilitated by the HIV/HBV co-infection, though different from Shuklaet.*et al's* suggestion [15] but was similar to what Nelson reported [16]. The marker for hepatic function (ALT) showed significant deterioration over the study period for those on no intervention. The laboratory results appeared to correspond to a pattern indicating a progression of both the HIV and HBV infections in the participants with no established form of intervention and may eventually lead to death if not controlled. This observation indicates that every case of HIV/HBV irrespective of the HIV stage may need treatment as early as possible.

When the slight positive change observed on the Se only group was compared to the negative change of 'no intervention group' Se, in the absence of any other intervention appeared to be beneficial, since it seemed to have slowed down the rate of HBV and HIV replication to an extent. In addition, marked increase in CD4 cell count over the intervention period seemed to correspond with some

improvement in the immune system following the use of Se. The hepatic function appeared stable in this group over the duration of intervention as shown by stable liver enzyme activities. This may indicate that Se is likely to have a protective effect on the hepatic cells and probably normalizes the liver enzymes. This supports some of the earlier reports of beneficial effect of Se on symptomatic hepatitis C patients with elevated aminotransferases when placed on a triple antioxidant therapy comprising alpha lipoic acid, Se and 'Silybummarianum'(milk thistle) [13]. Schwarz *et al* described the relationship between Se intake by food and prevention of liver necrosis in rats [14]. However Se alone may not stop HBV replication but may normalize the liver enzymes as such the findings probably suggest that every HIV/HBV co-infected person should be started on antiretroviral therapy irrespective of the level of CD4 cell count. Nigerian national guidelines for HIV/AIDS treatment and care in adolescents and adults (2005) did not recommend treatment for all HIV/HBV co-infected individuals. Since co-infection with HIV seems to speed up HBV disease progression, starting HIV treatment with HAART/Se earlier may delay or prevent liver damage from HBV. The fact that all (100%) participants on TG 1 & 2, who had undetectable HBV viral load at baseline, still remained undetected at 18th month; while only 68% of those who were not on HAART remained undetected at the end of the study, shows that there is need to keep monitoring all HBsAg positive patients periodically and probably place them on Se if mono-infected and baseline HBV viral load undetected.

CONCLUSION

Apart from enhancing CD4 cell count, selenium seems to have a protective effect on liver cells hence a resultant normalization of the liver enzymes in HBV infection. It is therefore concluded that selenium appears to have a beneficial effect as adjunct to HAART in management of HIV/HBV co-infection and may help to reduce development of liver cancer.

Recommendations: Selenium should be given as adjunct to HAART in management of HIV/HBV co-infection.

Limitation of the study: We were unable to assess the serum selenium level before and after intervention as well as other HBV markers due to financial constraints.

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