Liver enzyme activities in HIV seropositive pregnant women on Highly Active Antiretroviral Therapy (HAART)

Ikekpeazu Joy E[1], Ibegbu Madu D[1], Onyekwelu Kenechukwu C[1], Uche Ozichukwuamaka S[2]

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OBJECTIVES: This study was designed to evaluate the activities of liver enzymes in a cohort of HIV-positive pregnant women on highly active antiretroviral therapy (HAART).

METHODS: The activities of liver enzymes-ALP, ALT and AST- were assessed in ninety pregnant subjects aged 20-40 years at University of Nigeria Teaching Hospital (UNTH) Ituku-Ozalla Enugu. Thirty of the subjects were apparently healthy HIV seronegative pregnant women that served as the negative control; while sixty of the subjects were confirmed HIV infected subjects, thirty of which were on HAART (Test subjects); while thirty were not on HAART (positive control) and were further grouped into trimesters. Serum samples were prepared from blood samples collected from the subjects and assayed using standard methods.

RESULTS: The ALP, ALT and AST activities of the test subjects where compared with negative control in the first, second and third trimesters which showed significant increases in ALP ($p<0.001$,$p<0.05$ and $p<0.05$ respectively); ALT in all the trimesters were significantly different ($p<0.001$); while in AST significant differences could be observed in third trimesters $p<0.05$). When compared with positive control, ALP showed significant increase in all trimesters ($p<0.001$, $p<0.05$, and $p<0.001$). ALT was slightly but not significantly increased ($p>0.05$) in the second trimester, but significantly increased in first and third trimesters ($p<0.001$) but had no significant difference in the second trimester ($p>0.05$). The comparisons of negative and positive controls showed differences in enzyme activities in each of the trimesters.

CONCLUSION: Pregnancy, HIV infection, and drug treatment appear to have a cumulative effect though the treatment group had the highest level of increased ALT, AST and ALP activity across the trimesters.

Key Words: *Pregnant women; HAART; HIV; Serum enzymes; Enugu*

INTRODUCTION

The liver plays a pivotal role in the metabolism of most biomolecules. In addition to its many functions, it is the major excretory/detoxification organ in the body. Pregnancy is associated with diverse physiological changes required for foetal growth and development. Consequently, certain changes in values of liver function tests may occur during normal pregnancy. The abnormal liver function may be related to pregnancy or may co-exist with pregnancy. It may be divided into three major groups. The liver disorders specific to pregnancy include hyperemesis gravidarum, pre-eclampsia, HELLP (haemolysis, elevated liver enzymes, low platelets) syndrome, acute fatty liver of pregnancy and intra hepatic cholestasis of pregnancy (1,2). These are mostly trimester specific. The second group include intercurrent liver disease occurring in pregnancy such as viral hepatitis; Third group includes pregnancy with preexisting liver disease such as chronic active hepatitis, cirrhosis of liver etc (3). The liver diseases in pregnancy can have significant effect on the maternal and fetal outcome.

HAART is a term that refers to the use of the combination of three or more antiretroviral agents. Since its introduction, it has dramatically altered the treatment and life expectancy of Human Immunodeficiency Virus (HIV) patients for the better (4,5). In spite of the benefits of HAART, adverse effects, of which hepatotoxicity is a common finding (6).

Many factors have been associated with hepatic damage in HIV patients: antiretroviral treatment, co-infections with hepatitis B or C virus, opportunistic infections as cytomegalovirus, mycobacterium, leishmaniasis, or tumors (lymphoma and Kaposi's sarcoma), cholangitis associated to parasites (cryptosporidiosis and microsporidiosis) and toxicity related with non-antiretroviral drugs (trimetoprim and other antibiotics) (7). However chronic liver disease such as chronic hepatitis B or C, may lead to a rise in liver transaminases, which would make it difficult to distinguish the source of the enzyme increases.

Report has shown that severe hepatotoxicity in HIV-infected patients on HAART occurs in 5–10% of cases and the main risk factors are hepatitis co-infection, advanced liver disease, and elevated liver transaminases at the start of therapy. A number of antiretroviral drugs have been associated with increased risk of severe hepatotoxicity, which includes stavudine, didanosine, nevirapine, full-dose ritonavir and tipranavir (8).

All classes of antiretroviral therapies (ART) can induce liver toxicity but the probability and extent of injury varies substantially with the individual (9). It has been shown that patients on tipranavir suffered from transaminase elevations significantly more often than patients without it (8). Among the individual drugs, severe liver toxicity is more strongly associated with nevirapine (10). In a study reported 30% increase of transaminases because of nevirapine (NVP) (9). Hepatotoxicity reported as a major side effect of all antiretroviral classes with Nevirapine having the highest risk (11).

Hepatic toxicity occurs more frequently in women than in men and antiretroviral agents have been associated with liver toxicity. Some preliminary data suggest that the risk of hepatic toxicity with NVP may be higher in pregnant than non-pregnant women (10). In African women starting a nevirapine containing triple regimen in the 27th week of gestation has shown some levels of hepatotoxicity (12).

Nevirapine, a non-nucleoside reverse transcriptase inhibitor, is being increasingly used in pregnant patients owing to its favorable side effect profile and lower teratogenicity compared to protease inhibitors (9). However, hepatotoxicity is the major adverse effect of nevirapine (5%) and most often manifests as a hypersensitivity reaction with fever, rash and elevated liver tests within the first few weeks of therapy. Among the individual drugs, severe liver toxicity is more strongly associated with nevirapine (10). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are hepatic enzymes that could be used as markers of hepatocellular injury (13). Some cases of hepatotoxicity have been reported in pregnant women yet is not known whether pregnancy increases the risk of the occurrence of hepatotoxicity or rash (14-16). In the study of Nevirapine toxicity in HIV

[1]*Department of Medical Biochemistry, College of Medicine, University of Nigeria Enugu Campus (UNEC), Enugu, Nigeria;* [2]*Faculty of Health Sciences and Technology, Department of Medical Laboratory Sciences, University of Nigeria Enugu Campus (UNEC), Enugu, Nigeria*

Correspondence: Dr. Ibegbu Madu D, Department of Medical Biochemistry, College of Medicine, University of Nigeria Enugu Campus (UNEC), Enugu, Nigeria. Telephone +234(0)7053531358, e-mail: daniel.ibegbu@unn.edu.ng

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Infected Pregnant Women recorded some alterations in the ALT and AST in pregnant women on HAART (17).

The aim of this study was therefore to evaluate liver enzyme activities in different trimesters of pregnancy of HIV seropositive pregnant women treated with nevirapine-based HAART and compare the result with those of treatment-Naive patients and normal pregnancy.

MATERIALS AND METHODS

Ninety pregnant women at the University of Nigeria Teaching Hospital UNTH Ituku-Ozalla, Enugu State were recruited for the study. The subjects included those that showed laboratory evidence of HIV-infection with positive confirmatory tests and who were placed on highly active antiretroviral therapy (HAART). Subjects who were HIV seropositive but yet to be placed on HAART served as positive control while the negative control comprised of apparently healthy HIV seronegative pregnant volunteers. Informed consent was obtained from the subjects before commencement of the study. All the subjects recruited for this study were within the age range of 20-40 years.

Study design

The ninety pregnant women recruited for this study were grouped into three. The first group was the test group which comprised of thirty HIV positive pregnant women on HAART, the second group (positive control) were thirty HIV seropositive pregnant women but not on HAART, while the third group (negative control) comprised of thirty apparently healthy HIV seronegative pregnant women.

Blood sample collection

Blood samples were collected from each of the subjects by standard venepuncture at the antecubital vein. The samples were collected into already labeled plain test tubes and were allowed sufficient time to clot before centrifuging at 3000 rpm for 5mintes. The separated clear serum samples were transferred into sterile labeled bottles and were stored at -20°C and later used for the enzyme assays.

Enzyme estimation

Alkaline phosphatase estimation was measured using photometric method of King and Armstrong (18). The estimation of alanine amino transferase and aspartate amino transferase were measured by photometric method of Reitman and Frankel (19).

Statistical analysis

The data were analyzed using ANOVA, and when significant LDS was used as post-ANOVA test to determine level of significance at $p<0.05$ or $p<0.001$.

RESULTS AND DISCUSSION

HAART is the current treatment of choice for HIV infections; this is used to suppress viral multiplication by inhibition of viral enzymes and thus improvement of immune response which reduces opportunistic infections (Table 1 and Figure 1).

The current study investigated changes in liver enzymes during pregnancy – in normal, HIV infection and HAART therapy – the findings of this study show a significant ($p<0.001$) increase in ALT, AST and ALP across the groups in the trimesters (Table 2 and Figure 2).

The HIV seronegative pregnant (negative control group) showed significant increase in ALP activity ($p<0.001$), while ALP and AST showed significant level at ($p<0.05$) in the third trimester (Table 3 and Figure 3).

The enzyme elevation signals injury to liver cells and other cells of the body by the virus as well as the drugs used for treatment. The results are in agreement with previous findings that showed slight increases in ALT and AST in the second and third trimesters of normal pregnancy when compared with non-pregnant women though the values were below the upper normal limit (20-22). ALP has been shown to increase in third trimester of normal pregnancy due to the placental isoenzyme of ALP present during pregnancy (23). Liver toxicity including liver failure has been found to be associated with HAART treatment; though moderately significant elevations in ALT, AST and ALP activities have been found in asymptomatic seropositive HIV patients (24,25). Liver toxicity to be most frequent (30%) drug-related complication for patients on HAART who frequently visit hospital. Baseline elevation in serum aminotransferases was a significant risk factor for severe hepatotoxicity for all regimens (26,27). In a study in South African 17% of patients involved in the study receiving nevirapine had hepatotoxicity, greater increase in serum aminotransferase levels within the first 12 weeks; while

TABLE 1

Comparison of mean ± SD of liver ALP and ALT activities in pregnant HIV seropositive women on HAART (test group) and HIV seronegative pregnant women (negative control)

	First trimester			Second trimester			Third trimester		
Enzyme	Negative control (U/L)	Test group on HAART (U/L)	p-Value	Negative control (U/L)	Test group on HAART(U/L)	p-Value	Negative control (U/L)	Test group on HAART(U/L)	p-Value
ALP	46.2 ± 18.4	91.2 ± 12.2	p<0.001	92.4 ± 46.7	158.0 ± 92.1	p<0.05	103 ± 30.7	398.0 ± 187.0	p<0.05
ALT	4.47 ± 1.30	7.89 ± 1.88	p<0.001	5.24 ± 1.55	10.9 ± 2.04	p<0.001	8.40 ± 1.46	20.7 ± 7.74	p<0.001

TABLE 2

Comparison of Mean ± SD of liver ALP and ALT in HIV seropositive pregnant women on HAART (test group) and HIV seropositive pregnant women not on HAART (positive control)

	First trimester			Second trimester			Third trimester		
Enzyme	Positive control (Not on HAART) (U/L)	Test group on HAART (U/L)	p-Value	Positive control (Not on HAART) (U/L)	Test group on HAART(U/L)	p-Value	Positive control (Not on HAART) (U/L)	Test group on HAART(U/L)	p-Value
ALP	67.8 ± 20.6	91.2 ± 12.2	p<0.001	96.4 ± 13.8	158.0 ± 92.1	p<0.05	155.0 ± 56.1	398.0 ± 187.0	p<0.05
ALT	5.55 ± 1.67	7.89 ± 1.88	p<0.001	9.14 ± 1.72	10.9 ± 2.54	p>0.001	12.2 ± 3.01	20.7 ± 7.74	p<0.001

TABLE 3

Comparison of Mean ± SD of liver ALP and ALT in pregnant HIV seronegative women (negative control) and HIV seropositive pregnant women not on HAART (positive control)

	First trimester			Second trimester			Third trimester		
Enzyme	Negative control (U/L)	Positive control (Not on HAART) (U/L)	p-Value	Negative control (U/L)	Positive control (Not on HAART) (U/L)	p-Value	Negative control (U/L)	Positive control (Not on HAART) (U/L)	p-Value
ALP	46.2 ± 18.4	67.8 ± 20.6	p<0.05	92.4 ± 46.7	96.4 ± 13.8	p>0.05	103.0 ± 30.7	155.0 ± 56.1	p<0.05
ALT	4.47 ± 1.30	5.55 ± 1.67	p>0.05	5.24 ± 1.55	9.14 ± 1.72	p<0.001	8.40 ± 1.46	12.2 ± 3.10	p<0.001

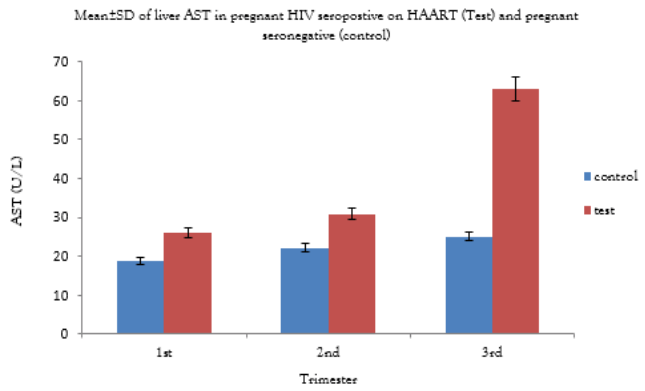

Figure 1) *The comparison of Mean ± SD of liver AST in pregnant HIV seropositive women on HAART (test group) and HIV seronegative pregnant women (negative control)*

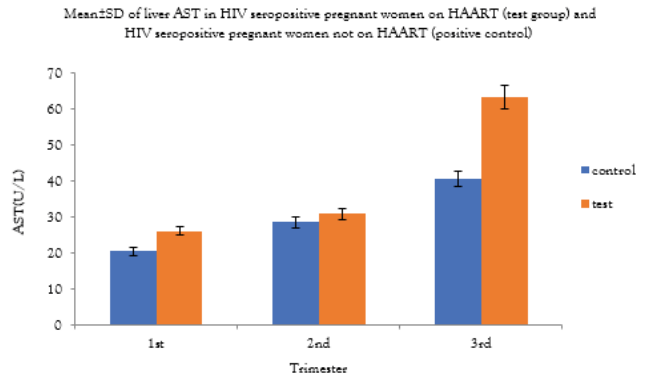

Figure 2) *Comparison of Mean ± SD of liver AST in HIV seropositive pregnant women on HAART (test group) and HIV seropositive pregnant women not on HAART (positive control)*

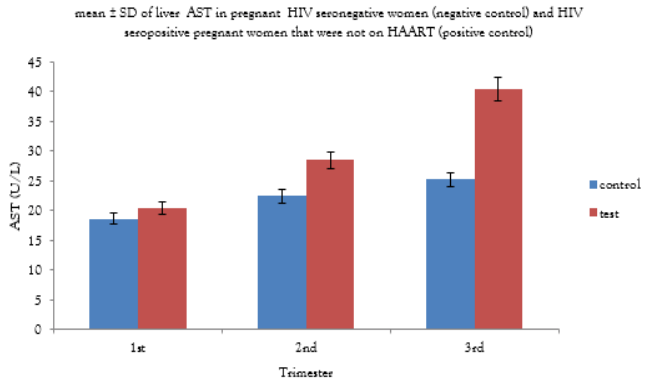

Figure 3) *Comparison of Mean ± SD of liver enzymes AST in pregnant HIV seronegative women (negative control) and HIV seropositive pregnant women not on HAART (positive control)*

fatal drug-related liver failure occurred in two women receiving nevirapine (28). Any damage to the liver causes medium elevations in the liver enzyme (ALT, AST and ALP) activities.

CONCLUSION

This study has shown that liver function is compromised only slightly in normal pregnancy but could increase significantly across the trimesters following HIV infection and treatment.

The steady rise in the levels of these enzymes following treatment may be an indication for change of therapy/regimen. Pregnancy, HIV infection, and drug treatment appear to have a cumulative effect though the treatment group had the highest level of increased ALT, AST and ALP activity across the trimesters.

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CONFLICTS OF INTEREST

The authors have declared no conflict of interest for this work.

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