Effect of highly active antiretroviral therapy (HAART) on some specific clotting profile in Human Immunodeficiency Virus-(HIV) positive pregnant women

Osime Odaburhine Evarista, Tijani Paul Ezimokhai, Blessing Airiagbonbu
Department of Medical Laboratory Science, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Nigeria

Address for correspondence:
Dr. Osime Odaburhine Evarista, Department of Medical Laboratory Science, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Nigeria. E-mail: evarista.osime@uniben.edu

Abstract

Background: In many developing countries with a significant proportion of human immunodeficiency virus (HIV)-positive patients are women of child-bearing age and would require antiretroviral therapy. This study aimed at evaluating the effect of highly active antiretroviral therapy (HAART) on some specific clotting profile in HIV-positive pregnant women. Subjects and Methods: This study comprised 150 patients consisting of 50 blood samples from pregnant women on HAART as test subjects, 50 pregnant HIV-positive women that were not on HAART as test subjects, and 50 pregnant HIV-negative women which served as controls. The test subjects were attending the prevention of mother-to-child transmission Clinic at the Central Hospital, Benin City. Specific clotting factors assayed were factors 11, V, V11, V111, 1X, X, X1, and X11. All were done using ELISA methods. Results: Factors 11 and V were reduced significantly in HIV-infected pregnant women on HAART and those not on HAART ($P < 0.05$) when compared with HIV-negative pregnant women. A significant increase in factors V11, V111, 1X, X, and X11 were observed in HIV-positive patients on HAART and those not on HAART when compared with HIV-negative pregnant women ($P < 0.05$). However, when HIV-positive patients on HAART were compared to HIV-positive women not on HAART, no statistical difference were observed ($P > 0.005$). Conclusion: There are changes in clotting profile of HIV-positive women on HAART and on those not on HAART and these changes are not due to the administration of antiretroviral therapy.

Key words: Clotting factors, highly active antiretroviral therapy, HAART, human immunodeficiency virus, pregnancy

INTRODUCTION

The human immunodeficiency virus (HIV) is one of the most important emerging infections. It is probably one of the diseases with multiple impacts on persons, families, communities, and the entire society. It is threatening especially in sub-Saharan African countries. In Nigeria, the prevalence rose from 3.8% in 1993 to 5.8% in 2001.[1]

Pregnancy has been observed to induce hyper coagulation which is likely an adaptive mechanism to reduce the risk of hemorrhage during and after the delivery process.[2] Thromboembolism is one of the leading causes of death associated with pregnancy occurring more in...
Recent theories have transitioned to a cell-based model in which both systems work together to form thrombin either on the surface of the site of vascular injury or on the surface of platelets. Antiretroviral therapy is largely employed in the prevention of mother-to-child transmission of the virus and these therapies are not without side effects. This work is, therefore, aimed at determining the effect of antiretroviral therapies on some specific clotting factors.

**SUBJECTS AND METHODS**

This study comprised 150 pregnant women, 50 pregnant HIV-positive women on highly active antiretroviral therapy (HAART) (as test subjects), 50 pregnant HIV-positive women that are not on HAART, and 50 pregnant HIV-negative women which served as controls. These subjects were attending the prevention of mother-to-child transmission (PMTCT) clinic at the Central Hospital Benin City. The recruitment process using the informed consent was initiated at the PMTCT clinic where HIV seropositive pregnant women are enrolled for the test group and the ANC for the control group over a period of 7 months. Verbal informed consent was obtained from each subject as well as ethical approval from the Ethics and Research committee of the Edo State Hospital Management Board. Furthermore, this study was carried out according to the principles of Helsinki Declaration.

Inclusion criteria were pregnant women confirmed HIV positive and had no previous history of coagulopathy, diabetic, or hypertensives, whereas exclusion criteria were pregnant women outside the age range, those that did not give consent and those not having other infections such as hepatitis B and C.

**Sample collection**

Five milliliters of blood was collected from each test and controls into sodium citrate anticoagulant specimen bottle in a concentration of 1 part of 0.11 M sodium citrate to 9 parts of whole blood.

**Laboratory analysis**

Extrinsic coagulation factors II, V, VII, X, and Intrinsic coagulation factors VIII, IX, XI, and XII were done using Hemostat ELISA reagents kits manufactured by Human Gesellschaft for Biochemica and Diagnostic mbHMaxplanck-Ring 21-D-65205, Wiesbaden Germany. They were done separately following the manufacturer's procedure.

**Statistical analysis**

Data obtained were analyzed using Statistical Package for the Social Sciences (version 17, SPSS Inc., Chicago, Illinois, USA) and expressed in mean ± standard error of the mean (SEM). Student's t-test and analysis of variance were used in comparing values between and among mean of groups, respectively. Values of $P < 0.05$ at 95% confidence limit (CL) was considered statistically significant, whereas $P > 0.05$ at 95% CL was considered nonsignificant.

**RESULTS**

This showed that the HIV-positive pregnant women on HAART had a significantly higher age difference when compared to HIV-positive women not on HAART and pregnant negative women. Eighty percent of the test subjects were on zidovudine, lamivudine, nevirapine, combination (AZT + 3TC + TDF), 10% were on tenofovir and efavirenz combination (TDF + EFV) and 10% were on tenofovir, nevirapine, and efavirenz (TDF + 3TC + EFV) combination. All the test subjects were on HAART for a minimum of 3 years. The mean ± SEM of their gestational ages were $22.12 ± 1.98$, $19.80 ± 1.46$, and $18.22 ± 1.04$ weeks, as shown in Table 1. Factors II and V reduced significantly, in test subjects when compared with the controls ($P = 0.037$, $P = 0.003$), while factors VII, X, and VIII increased significantly in test when compared to controls ($P = 0.008$, $P = 0.004$, and $P = 0.0001$, respectively). Furthermore, FIX and FXII increased significantly in test when compared to the controls ($P = 0.007$, $P = 0.0007$), respectively, as shown in Tables 2 and 3. Although there were variations in comparison of the clotting factors of HIV-positive pregnant women on HAART, those not on HAART, these variations were not statistically significant as shown in Table 4.

**Key:** AZT-AZT: Azidothymidine, now renamed zidovudine, but still best known by the abbreviation AZT.
- $3\text{T C} = \text{L a m i v u d i n e } \left( [ - ] - \text{L} - L \quad \text{dideoxy-3'-thiacytidine} \right)$
- TDF: Tenofovir disoproxil fumarate
- EFV: Efavirenz.
DISCUSSION

There exist abnormalities in coagulation factors in HIV-positive patients and those on antiretroviral therapy. Moreover, these changes may be exacerbated by pregnancy states which may contribute to thrombosis or hemorrhage, against this was this study carried out to examine the effect of antiretroviral therapy on some specific clotting factors in HIV-positive pregnant women on these drugs. In this study, there was an increase in the mean ages and blood pressure of HIV-positive pregnant women on HAART and those not on HAART when compared to HIV-negative pregnant women. This may be attributed to some psychological feeling and apprehension.

Table 1: Some demographic indices of test and controls

| Parameters | HIV positive pregnant women on HAART (n=50) | HIV positive pregnant women not on HAART (n=50) | HIV negative pregnant women (n=50) | P       | Level of significance |
|------------|-------------------------------------------|-----------------------------------------------|-----------------------------------|---------|-----------------------|
| Age (years) | 34.50±0.709                               | 32.60±0.415                                   | 31.40±0.416                       | 0.000   | Highly significant    |
| Medical history (clotting disorders) | None (n=50; 100%) | None (n=50; 100%) | None (n=50; 100%) | -       | -                     |
| Use of HAART | All (n=50; 100%) | None | None | -       | -                     |
| Other drugs | Cotrim (n=50, 100%) | Cotrim (n=50, 100%) | None | -       | -                     |
| Mean BP (mm/Hg) | Systolic=134.70±2.786 | Systolic=128.68±1.714 | Systolic=121.04±2.1479 | 0.0003  | Highly significant    |

Table 2: Mean±standard error of the mean of specific clotting factors of the test and controls

| Parameters | HIV-Positive pregnant women on HAART (n=50) | HIV-positive pregnant women not on HAART (n=50) | HIV-negative pregnant women (n=50) | P           | Level of significance |
|------------|-------------------------------------------|-----------------------------------------------|-----------------------------------|--------------|-----------------------|
| Factor II (IU/ml) | 0.75±1.83 | 0.78±0.22 | 0.95±0.7 | 0.037 | Significant |
| Factor V (IU/ml) | 0.50±0.20 | 0.51±0.61 | 0.98±1.26 | 0.0003 | Highly significant |
| Factor VII (IU/ml) | 1.26±1.07 | 1.19±2.47 | 0.89±2.15 | 0.008 | Significant |
| Factor X (IU/ml) | 1.45±0.09 | 1.47±0.002 | 1.18±0.261 | 0.004 | Significant |
| Factor VIII (IU/ml) | 1.53±1.01 | 1.57±0.07 | 0.84±1.56 | 0.001 | Highly significant |
| Factor IX (IU/ml) | 1.31±0.031 | 1.36±0.062 | 1.14±0.012 | 0.007 | Significant |
| Factor XII (IU/ml) | 1.39±1.12 | 1.29±11.6 | 0.86±0.13 | 0.0007 | Highly significant |

Table 3: Mean±standard error of the mean of clotting profile of human immunodeficiency virus positive women on highly active antiretroviral therapy and human immunodeficiency virus negative pregnant women

| Parameters | HIV-positive women on HAART | HIV-negative pregnant women | P           | Level of significance |
|------------|-----------------------------|-----------------------------|--------------|-----------------------|
| Factor II (IU/ml) | 0.75±1.83 | 0.95±0.7 | 0.037 | Significant |
| Factor V (IU/ml) | 0.50±0.20 | 0.98±1.26 | 0.0003 | Highly significant |
| Factor VII (IU/ml) | 1.26±1.07 | 0.89±2.15 | 0.008 | Significant |
| Factor VIII (IU/ml) | 1.53±1.01 | 0.84±1.56 | 0.001 | Highly significant |
| Factor IX (IU/ml) | 1.31±0.031 | 1.14±0.012 | 0.007 | Significant |
| Factor XII (IU/ml) | 1.39±1.12 | 0.86±0.13 | 0.0007 | Highly significant |

Table 4: Mean±standard error of the mean of some clotting profile of human immunodeficiency virus positive pregnant women on highly active antiretroviral therapy and human immunodeficiency virus positive pregnant women not on highly active antiretroviral therapy

| Parameters | HIV-positive women on HAART | HIV-positive women not on HAART (n=50) | P       | Level of significance |
|------------|-----------------------------|----------------------------------------|---------|-----------------------|
| Factor II (IU/ml) | 0.75±1.83 | 0.78±0.22 | 0.411 | Not significant |
| Factor V (IU/ml) | 0.50±0.20 | 0.51±0.61 | 0.628 | Not significant |
| Factor VII (IU/ml) | 1.26±1.07 | 1.19±2.47 | 0.460 | Not significant |
| Factor VIII (IU/ml) | 1.45±0.09 | 1.47±0.002 | 0.711 | Not significant |
| Factor IX (IU/ml) | 1.53±1.01 | 1.57±0.07 | 0.518 | Not significant |
| Factor XII (IU/ml) | 1.39±1.12 | 1.29±11.6 | 0.103 | Not significant |

HIV=Human immunodeficiency virus; HAART=Highly active antiretroviral therapy; BP=Blood pressure
encountered following the outcome of their status as well as social stigmatization associated with such disease states.[6] It was observed that HIV-infected pregnant women on HAART and those not on HAART had a significant lower factor II and FV values when compared with the controls ($P < 0.003$). The interaction of HIV with the liver which is the primary site in the production of factor II and V may be responsible for this decrease.[9]

There was a significant increase in factors VII, VIII, and IX in HIV-positive women on HAART and those not on HAART when compared to the HIV-negative pregnant women ($P < 0.05$). This may be attributed to the nonproduction of inhibitors to some coagulation factors,[10] especially as all the patients used in this study were hepatitis C negative which was one of the exclusion criteria for this study.

On comparison of the studied parameters in HIV-positive pregnant women on HAART and those not on HAART, alterations were observed in these parameters but were not statistically significant. This shows that HAART may not be directly contributory to these changes but the virus itself or the pregnancy states as observed by other studies.[11,12] Opportunistic infections, related malignancies, acquired hypercoagulable state, and endothelial dysfunction have been attributed to a lot of these changes in pregnancy and HIV infections.[13,14] Furthermore, antiretroviral drugs containing protease inhibitors are proposed to cause endothelial dysfunction by their effects on the metabolism of lipid and glucose.[15] Factors II and V were significantly reduced in HIV-positive pregnant women when compared to HIV-negative pregnant women. This may predispose these women to hemorrhage during delivery. This also is in line with other studies.[14] While factors VII, VIII, X, IX, and XII were significantly increased ($P < 0.05$) in HIV-positive pregnant women on HAART than HIV-negative pregnant women. The hypercoagulability of blood during pregnancy has been confirmed with TEG and is thought mainly due to the increased production of factor VII and fibrinogen. This agrees with the finding of Katz and Beilin.[2]

Although HIV-infected patients are at higher risk for VTE, little work has been done on defining the exact mechanisms by which this phenomenon occurs and still less has been done on examining the role thromboprophylaxis in HIV-infected individuals. Notably, the 2008 American College of Chest Physicians guidelines on antithrombotic and thrombolytic therapy are silent on these events.[16] Furthermore, there are some important concerns about the therapy of HIV-related thromboses. VTE in women during pregnancy and puerperium, has been described in the literature with an incidence of approximately 1–2 in 1000 pregnancies. Women are five times having a greater chance of developing VTE during pregnancy or puerperium compared to nonpregnant. A recent study reported the annual incidence of VTE in HIV-positive women during puerperium of 313/1000 persons-years (95% CL 65–915). According to this finding, HIV-positive pregnant women are 120-fold more likely to develop VTE than HIV-negative controls,[12] whereas the risk is 157-fold higher compared to HIV-negative pregnant women.[18] This is, however, at variance with this study which shows a greater risk of hemorrhage than thromboses.

**CONCLUSION**

Results obtained showed that HIV-positive pregnant women on HAART and those not on HAART had a significantly lower factor II and IV values when compared to HIV-negative pregnant women. Furthermore, a significant increase in factors VII, VIII, IX, X, and XII were obtained in HIV-positive pregnant women on HAART and those not on HAART when compared to HIV-negative pregnant women. However, when HIV-positive women on HAART were compared with those not on HAART, no significant difference was observed. These observations, therefore, show that there are alterations in specific clotting factors in HIV-positive pregnant women, but these changes may be attributed to the HIV status and not the administration of antiretroviral treatment.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

**REFERENCES**

1. Ibeh BO, Omodamiro DO, Ibeh U, Habu BU. Biochemical and haematological changes in HIV subjects receiving winniecure antiretroviral drugs in Nigeria. J Biomed Sci 2013;20:20–73.
2. Katz D, Beilin Y. Disorders of coagulation in pregnancy. Br J Anaesth 2015;10:374–91.
3. Nasir IA, Owolagba A, Ahmad AE, Barma MM, Musa Po PO, Bakare M, et al. Effects of first-line anti-retroviral therapy on blood coagulation parameters of HIV-infected patients attending a tertiary hospital at Abuja, Nigeria. Malays J Pathol 2016;38:103–9.
4. de Andrade CM, Duarte G, Quintana SM, Montes MB, Toloi MR. Effect of antiretroviral therapy on hemostasis in Brazilian pregnant women with HIV infection. Blood Coagul Fibrinolysis 2007;18:769–74.
5. Eyal A, Veller M. HIV and venous thrombotic events. S Afr J Surg 2009;7:93–5.
6. Guidozzi F, Black V. The obstetric face and challenge of HIV/AIDS. Clin Obstet Gynecol 2009;52:270–84.
7. Polderman H. Hypothermia and coagulation. Crit Care 2012;16:20–7.
8. Ujah IA, Aisien OA, Mutihir JT, Vanderjagt DJ, Glew RH, Uguru VE. Factors contributing to maternal mortality in north-central Nigeria: A seventeen-year review. Afr J Reprod Health 2005;9:27–40.
9. Ope J. Haematological complications of HIV infection. S Afr Med J 2011;10:2-6.
10. Sturt AS, Dokubo EK, Sint TT. Antiretroviral therapy (ART) for treating HIV infection in ART-eligible pregnant women. Cochrane Database Syst Rev 2010: DOI: 10.1002/14651858.CD008440 CD008440.
11. Klein SK, Slim EJ, de Kruijf MD, Keller TT, ten Cate H, van Gorp EC, et al. Is chronic HIV infection associated with venous thrombotic disease? A systematic review. Neth J Med 2005;63:129-36.
12. Ramogale MR, Moodley J, Sebiloane MH. HIV-associated maternal mortality-primary causes of death at King Edward VIII Hospital, Durban. S Afr Med J 2007;97:363-6.
13. Osime OE, Eze-Onakwurh JU, Kolade SO. Fibrinolytic changes in pregnant women on highly active antiretroviral therapy. Saudi Med J 2015;36:200-3.
14. Thulasi Raman R, Manimaran D, Rachakatla P, Bharathi K, Afroz T, Sagar R. Study of basic coagulation parameters among HIV patients in correlation to CD4 counts and ART status. J Clin Diagn Res 2016;10:EC04-6.
15. Thornton P, Douglas J. Coagulation in pregnancy. Best Pract Res Clin Obstet Gynaecol 2010;24:339-52.
16. Hirsh J, Guyatt G, Albers GW, Harrington R, Schünemann HJ. Antithrombotic and thrombolytic therapy: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). Chest 2008;133:110S-2S.
17. Pham PA, Flexner C. Emerging antiretroviral drug interactions. J Antimicrob Chemother 2011;66:235-9.
18. Sullivan PS, Dworkin MS, Jones JL, Hooper WG. Epidemiology of thrombosis in HIV infected individuals. AIDS 2000;14:321-4.