

Fibrinolytic changes in pregnant women on highly active antiretroviral therapy

Odaburbine E. Osime, PhD, Joseph U. Ese-Onakewhor, MBBS, FWCOG, Samson O. Kolade, BMLS.

ABSTRACT

الأهداف: تقييم نشاط الفيبرينوليتيك لدى مجموعة من النساء المصابات بنقص المناعة المكتسب واللاتي يخضعن لعلاج مكثف ضد فيروسات النسخ العكسي.

الطريقة: لقد قمنا بتجميع عينات الدم من 50 مريضة مصابة بفيروس نقص المناعة المكتسبة واللاتي يخضعن لعلاج ضد فيروسات النسخ العكسي (مجموعة الدراسة)، بالإضافة إلى 50 مريضة مصابة بالفيروس ولكنها غير خاضعة لعلاج ضد فيروسات النسخ العكسي (مجموعة الشاهد). ولقد كانت هذه العينة تتراد عيادة وقاية انتقال فيروس نقص المناعة من الأم إلى الجنين، جامعة مستشفى بينين الأكاديمي، بينين، نيجيريا وذلك خلال الفترة من يناير إلى يونيو 2014م. لقد قمنا باستخدام الكتيب الإرشادي المعياري وذلك من أجل قياس كلاً من: تركيز الفيبرينوليتيك في البلازما، واختبار انحلال الغلوبين الحقيقي، وحجم الخلايا المكدسة، ولزوجة الدم.

النتائج: أشارت النتائج لدى مجموعة الدراسة أن كلاً من معدل والخطأ المعياري لمعدل تركيز الفيبرينوليتيك في البلازما قد وصل إلى $4.02 \pm 0.13 \text{g/l}$ ، فيما وصل اختبار انحلال الغلوبين الحقيقي إلى 378 ± 15 دقيقة. ولقد كانت هذه النتائج عالية بشكل واضح من الناحية الإحصائية لدى مجموعة الدراسة عند مقارنتها بمجموعة الشاهد ($p < 0.05$) (تركيز الفيبرينوليتيك في البلازما $29.1 \pm 0.38\%$ ، واختبار انحلال الغلوبين الحقيقي $3.46 \pm 0.12 \text{g/l}$ دقيقة). فيما وصلت نتائج حجم الخلايا المكدسة لدى مجموعة الدراسة إلى $29.1 \pm 0.38\%$ ، وقد كان ذلك أقل بصورة واضحة من الناحية الإحصائية من مجموعة الشاهد ($p > 0.05$) ($31.3 \pm 0.43\%$). ووصلت لزوجة الدم بين مجموعة الدراسة إلى $1.76 \pm 0.02 \text{ mPa/s}$ ، فيما كانت أعلى لدى مجموعة الشاهد ($1.73 \pm 0.02 \text{ mPa/s}$)، غير أن هذا الفرق بين المجموعتين لم يكن واضح من الناحية الإحصائية ($p > 0.05$). ولقد كان هناك فروق بين المتغيرات المختلفة التي تضمنتها الدراسة وذلك عند المقارنة فيما بينها، فيما لم يكن لهذه الاختلافات نمطاً محدداً.

الخاتمة: أظهرت الدراسة بأن لدى العلاج المكثف ضد فيروسات النسخ العكسي القدرة على تغيير نشاط الفيبرينوليتيك، وأن هذا يمكن أن يعرض المرأة الحامل إلى خطر الإصابة بفقر الدم وفرط فبرينوجين الدم.

Objectives: To report on the changes in fibrinolytic activity in human immunodeficiency virus (HIV) infected pregnant women who are undergoing highly active antiretroviral therapy (HAART).

Methods: Blood was collected from 50 HIV positive women on HAART (test subjects), and 50 HIV positive women not on HAART (controls). These women were attending the prevention of mother to child clinic (PMTCT) of the University of Benin Teaching Hospital, Benin City, Nigeria from January to June 2014. Standard manual techniques were used to estimate plasma fibrinogen concentration (PFC), euglobulin lysis time (ELT), packed cell volume (PCV), and plasma viscosity (PV).

Results: The mean \pm standard error of mean (SEM) of PFC was $4.02 \pm 0.13 \text{g/l}$ and ELT from the test subjects was 378 ± 15 mins was significantly higher ($p < 0.05$) compared with the control subjects (PFC $3.46 \pm 0.12 \text{g/l}$ and ELT 267 ± 9.0 mins). The PCV or hematocrit values in the test subject was $29.1 \pm 0.38\%$, which was significantly lower ($p < 0.05$) compared with the control subject ($31.3 \pm 0.43\%$). The PV in the test subject was $1.76 \pm 0.02 \text{ mPa/s}$, while the control subjects was higher ($1.73 \pm 0.02 \text{ mPa/s}$). This increase was not statistically significant ($p > 0.05$). There were differences in the various parameters investigated when the various trimesters were compared. These differences did not, however, follow a particular pattern.

Conclusion: Highly active antiretroviral therapy can cause changes in fibrinolytic activity that may predispose pregnant women to hyperfibrinogenemia and anemia.

Saudi Med J 2015; Vol. 36 (2): 200-203
doi: 10.15537/smj.2015.2.10372

From the Department of Medical Laboratory Science (Osime, Kolande), and the Department of Obstetrics and Gynecology (Ese-Onakewhor), University of Benin Teaching Hospital, Benin City, Nigeria.

Received 15th October 2014. Accepted 22nd December 2014.

Address correspondence and reprint request to: Dr. Odaburbine E. Osime, Department of Medical Laboratory Science, School of Basic Medical Science, College of Medical Science, University of Benin, Benin City, Nigeria. E-mail: clementosime@yahoo.com

During pregnancy the physiology of a woman is temporary changed to accommodate the newly developing fetus.¹ Conception occurs during ovulation, which is approximately on the fourteenth day of a regular menstrual cycle. In conception, the ovum is fertilized in the fallopian tube and becomes a zygote, which is then carried into the uterus. There are changes in the coagulation and fibrinolytic system during pregnancy and knowledge of these physiological changes characterized by hemodilution, changes in the concentration of one or more plasma protein fractions, and reduced fibrinolytic activity is necessary to manage 2 of the more serious problems in pregnancy; namely hemorrhage and thrombo-embolic diseases.² Increased levels of plasma proteins and reduced fibrinolytic activity have been reported in pregnancy,³ while prolonged euglobulin lysis time (ELT) and increased levels of fibrinogen have also been observed in pregnancy.⁴ While numerous studies have examined optimal methods for prevention of mother to child transmission of human immunodeficiency virus (HIV) and subsequent response to HAART,⁵ as well as the impact of pregnancy on outcomes of HIV in the pre-HAART era,⁶ little is known of the impact of pregnancy on response to HAART in Africa. There are several biologically plausible mechanisms that might alter efficacy of several antiretroviral agents including changes in enzyme activity and beta-estradiol levels, pregnancy-related changes in blood volume and body mass index, and factors which may compromise adherence to HAART ante and post-partum, such as nausea/vomiting, labor-associated morbidity, or responsibility for a new infant.⁷ Human immunodeficiency virus infected patients have been reported to be at risk of cardiovascular diseases, (CVD) mostly due to the use of antiretroviral agents.^{8,9} Protease inhibitors have been mostly implicated.¹⁰ All these are also connected to fat redistribution dyslipidemia and insulin resistance observed in HIV patients;^{11,12} thus, this study aims to report on the changes in fibrinolytic activity in HIV infected pregnant women who are undergoing HAART.

Methods. The study comprised 100 subjects consisting of 50 samples from pregnant women on

Disclosure. Authors have no conflict of interests, and the work was not supported or funded by any drug company.

HAART (Zidovudine and lamivudine) (test subjects), and 50 from pregnant women that are not on HAART which served as controls. The HIV patients were attending the prevention of mother to child transmission (PMTCT) clinic at the University of Benin Teaching Hospital, Benin City, Nigeria, from January to June 2014. Verbal informed consent was obtained from all subjects as well as ethical approval from the Ethics and Research Committee of the University of Benin Teaching Hospital. Also, this study was carried out according to the principles of Helsinki declaration.

The controls were matched with the HIV patients for age and gender. Inclusion criteria were pregnant women, confirmed HIV positive, and not diabetic or hypertensive. While exclusion criteria were non-pregnant women and HIV negative.

Whole blood samples were drawn with minimum stasis into 5ml bottles via the ante-cubital vein using a disposable plastic syringe and needle. Each sample was then mixed gently and thoroughly to ensure anticoagulation, and to prevent cell lysis. They were immediately taken in insulated flasks to the laboratory of the University of Benin Teaching Hospital.

Hematocrit values were estimated manually, while the plasma fibrinogen concentration (PFC) was estimated using the Ingram¹² method. The micro hematocrit tubes were approximately three-quarters of their length filled with anticoagulated blood samples and sealed on one end. Following centrifugation for 5 mins at 12,000 g (Hawksley, Hematocrit Centrifuge; Hawksley, England), the hematocrit values was read with the Hawksley hematocrit reader. One milliliter of test plasma was prewarmed at 37°C and calcium chloride added and incubated at 37°C for 30 mins. The adherent fibrin was removed, washed, and allowed to dry carefully on Whatman No. 1 filter paper. After drying, the clot was weighed and the concentration of fibrinogen was calculated.

A modification of the method of Reid and Ugwu¹³ was used. A 1 ml syringe with a hypodermic needle (21.6 x 0.8 x 4 mm) was used. Plasma was drawn into the syringe, avoiding bubbles until the 1.0 ml was mark. The plunger was carefully removed, and the carrying time for the entire plasma to drain was noted. This was carried out twice for each sample, and the average was taken for that sample. The entire process was repeated using distilled water. The plasma viscosity is the ratio of the flow rate of plasma to water. The ELT was carried out using the method described by Omoigberale et al.¹⁴ To 9.5 ml of 1% acetic acid in a test tube, 0.5ml of plasma was added and the tube kept at 4°C for 30 mins to precipitate the euglobulin fraction. The tube was then centrifuged at 2000 rpm for 10 mins. The supernatant

was discarded and the tube was inverted to get rid of acetic acid. The deposit was reconstituted with 0.5 ml borate buffer (sodium borate 1 g, sodium chloride 9 g, and distilled water 100 ml). The tube was pre-warmed at 37°C alongside calcium chloride (0.025N) for 2 mins, and 0.5 ml of the calcium chloride was added to the tube containing the deposit and borate buffer. A stop watch was started. Immediately after a clot was observed and the carrying time for the euglobulin fraction to lyse completely was recorded in minutes.

The statistical analysis was carried out using the Statistical Package for Social Sciences version 16.0 (SPSS INC., Chicago, Illinois, USA). The data were analyzed using unpaired student t-test. The results were summarized as mean \pm SEM, at 95% confidence limit.

Results. From the data obtained, the ages of the subjects ranges from 21-38 years. There was a significant increase ($p < 0.001$) in PFC, and ELT between pregnant women on HAART (test subjects)

Table 1 - Mean and standard error of mean between test and control subjects in 100 HIV subjects.

Parameters	Test	Control	P-value
	Mean \pm SEM	Mean \pm SEM	
PCV%	29.1 \pm 0.38	31.3 \pm 0.43	<0.0001
ELT (mins)	378 \pm 15	267 \pm 9.0	<0.0001
PFCg/l	4.02 \pm 0.13	3.56 \pm 0.12	0.002
PV mpa/s	1.76 \pm 0.02	1.73 \pm 0.02	0.371

PCV - packed cell volume, ELT - euglobulin lysis time, PFC - plasma fibrinogen concentration, PV - plasma viscosity, HIV - human immunodeficiency virus

Table 2 - Fibrinolytic changes between test and control subjects in the first to third trimester of pregnancy in 100 HIV subjects.

Parameters	Test	Control	P-value
	Mean \pm SEM	Mean \pm SEM	
<i>First trimester</i>			
PCV (%)	29.5 \pm 0.909	30.1 \pm 0.966	0.6359
ELT (mins)	381 \pm 35.1	243 \pm 15.5	0.0009
PFC (g/l)	4.11 \pm 0.216	3.48 \pm 0.201	0.0432
PV (mPa/s)	1.79 \pm 0.031	1.72 \pm 0.031	0.117
<i>Second trimester</i>			
ELT (mins)	368 \pm 20.1	283 \pm 11.8	0.0007
PFC (g/l)	3.90 \pm 0.187	3.63 \pm 0.187	0.3206
PCV (%)	29.1 \pm 0.464	31.2 \pm 0.492	0.0038
PV (mPa/s)	1.75 \pm 0.023	1.74 \pm 0.021	0.8344
<i>Third trimester</i>			
ELT (mins)	401 \pm 29.9	259 \pm 22.4	0.0014
PFC (g/l)	4.21 \pm 0.288	2.96 \pm 0.222	0.0031
PCV (%)	28.7 \pm 0.920	33.2 \pm 0.772	0.0015
PV (mPa/s)	1.74 \pm 0.0370	1.74 \pm 0.040	0.9197

PCV - packed cell volume, ELT - euglobulin lysis time, PFC - plasma fibrinogen concentration, PV - plasma viscosity, HIV - human immunodeficiency virus

when compared with pregnant women not on HAART (controls). The PCV decreased significantly ($p < 0.001$) between test when compared with the control while the PV did not show any significant difference when compared ($p > 0.05$) (Table 1). There was a significant decrease in ELT and PFC between test and control subjects in the first trimester, while the PCV and PV did not show any significant change ($p > 0.05$) in the first trimester of pregnancy (Table 2). The ELT and PCV were significantly reduced between test and control as pregnancy progressed while the PFC and PV did not show significant change. In the third trimester of pregnancy, ELT and PFC reduced significantly ($p < 0.05$) between the control and test subjects while the PCV of the controls increased when compared with the test ($p < 0.05$). There were no significant differences in the PV in the last trimester of pregnancy ($p > 0.05$).

Discussion. The intense effect of HIV on the world is in irrefutable.¹⁵ To fight viral strain alterations, HAART has increased in intricacy and effectively decreased deaths from opportunistic infection in those that are candidates for this treatment. However, these advances are tainted with metabolic long-term side effects. Some of which are directly attributed to HIV protease inhibitors.¹⁶ In this study, HIV infected pregnant women on HAART had a significantly lower PCV value compared with the controls ($p < 0.0001$). In HIV negative pregnant women, PCV has been observed to reduce due to increasing demand by the growing fetus. Human immunodeficiency virus infection and the therapy may further reduce PCV in such individuals, which may subsequently lead to anemia if not properly monitored. This observation is in line with earlier reports.¹⁷ The PCV value affects erythrocyte sedimentation rate, which in turn affects erythrocyte aggregation and ultimately blood flow.¹⁸ Thus, HIV patients may be at risk of CVD.

There was a significant increase in ELT, PFC, and PV ($p < 0.001$) in this study. The increase in plasma viscosity observed among HIV pregnant women on HAART in this study correlates with the observed increase in plasma fibrinogen concentration and invariably increases in ELT. Fibrinogen concentration significantly affects plasma viscosity. These findings agree with previous reports.^{18,19} It has been reported that increase in fibrinogen concentration, which would invariably lead to increase in plasma viscosity is a risk factor for CVD. Increase in serum viscosity has a similar effect.² Both plasma and serum viscosity are useful indicators of acute inflammation. An increase in inflammatory proteins such as haptoglobin, C-reactive

proteins, and immunoglobulin produced against the HIV virus or any other opportunistic infection associated with HIV, cause an increase in serum viscosity. Euglobulin lysis time, PFC, and PV were observed to reduce in the second and third trimester of pregnancy (Table 2). This could be attributed to the side effects of HAART on these individuals some of which may be directly connected to HIV protease inhibitors. Several studies have linked HIV protease inhibitors with the activation of endoplasmic reticulum stress and oxidative stress as well as an increase in inflammatory cytokine production from several cell types including macrophages, hepatocytes, intestinal epithelial cells, and adipocytes.¹⁶ However, the underlying cellular and molecular mechanisms remain to be fully identified and therapeutic strategies are currently unavailable.

In conclusion, HAART can cause changes in fibrinolytic activity, which may predispose pregnant women to hyperfibrinogenemia and anemia. Even though the life expectancy of HIV-infected patients under HAART has been extended, the various HAART-induced side effects significantly affect quality of life. Therefore, treatment of HIV infection requires the balance between the beneficial effects of viral suppression and the drug-induced toxic effects.

Acknowledgment. We are grateful to the authorities of the University of Benin Teaching Hospital, Benin City, Nigeria especially to the staff of the Prevention of Mother to Child Transmission (PMTCT) Clinic for their assistance in data collection.

References

- Lau G. Are maternal deaths on the ascent in Singapore? A review of maternal mortality as reflected by coronial casework from 1990 to 1999. *Ann Acad Med Singapore* 2002; 31: 261-272.
- Koos JB, Moore JP. Maternal Physiology during pregnancy. In: Alan HD, Lauren NC, editors. Current obstetric and gynaecologic diagnosis and treatment. 9th ed. New York (NY): McGraw Hill Companies; 2003. p. 154-162.
- Akinsete I, Uyanwah PO. The fibrinolytic enzyme system in pregnancy in Nigerians. *Afr J Med Sci* 1989; 18: 89-93.
- Adediran IA, Durosini MA, Ogunniyi SO, Akinda NO, Akanmu AS. Haemostatic parameters in normal pregnant Nigerian and Nigerians with hypertensive disorder of pregnancy. *Niger Postgr Med T* 1999; 6: 49-52.
- Jourdain G, Ngo-Giang-Huong N, Le Coeur S, Bowonwatanuwong G, Kantypong P. Intrapartum exposure to nevirapine and subsequent maternal responses to nevirapine-based antiretroviral therapy. *N Engl J Med* 2004; 51: 229-240.
- Lieve BP, Shafer LA, Mayanja BN, Whitworth JA, Grosskurth H. Effect of pregnancy on HIV disease progression and survival among women in rural Uganda. *Trop Med Int Health* 2007; 12: 920-928.
- Hadigan C1, Meigs JB, Rabe J, D'Agostino RB, Wilson PW, Lipinska I, et al. Increased PAI-1 and tPA antigen levels, are reduced, with metformin therapy in HIV-infected patients with fat redistribution and insulin resistance. *T. Clin Endocrinol Metab* 2001; 86: 939-943.
- Andrade ACO, Cotter BR. Endothelial Function and Vascular diseases in HIV infected patient. *Braz J Infect Dis* 2006; 10: 139-145.
- Barbaro G. HIV infection, highly active antiretroviral therapy and the cardiovascular system. *Cardiovascular Res* 2003; 111: 389-397.
- Carr A, Samaras K, Thorisdottir A, Kauffman GR, Chisolm DJ, Cooper DA. Diagnosis, prediction and natural course of HIV-1 protease-inhibitor associated lipodystrophy, hyperlipidemia, and diabetes mellitus; a cohort study. *Lancet* 1999; 353: 2093-2099.
- Hadigan C, Miller K, Corcoran C, Anderson E, Basgoz N, Grinspoon S. Fasting hyperinsulinemia and changes in regional body composition in HIV-infected women. *J Clin Endocrinol Metab* 1999; 84: 1932-1937.
- Ingram GI. A suggested schedule for the rapid investigation of acute haemostatic failure. *J Clin Pathol* 1961; 14: 356-360.
- Reid A, Ugwu AC. A simple technique of rapid determination of plasma and whole blood viscosity. *Nig J Physiol Sci* 1989; 3: 45-48.
- Omoigberale AI, Abiodun PO, Famodu AA. Fibrinolytic activity in children with Plasmodium falciparum Malaria. *East Afr Med J* 2005; 82: 104-106.
- Zha BS, Studer EJ, Zha H, Hylemon PB, Pandak W, Zhou H. Highly active antiretroviral therapy. In: Tang YW. Recent Translational Research in HIV/AIDS. Rijeka (HR): InTech; 2011.
- Moyle G. Anaemia in persons with HIV infection prognostic marker and contributor to morbidity. *AIDS Rev* 2002; 4: 13-20.
- Mchedlishvili G, Varazashvili M, Gobejishvili L. Local RBC aggregation disturbing blood fluidity and causing stasis in microvessels. *Clin Hemorheol Microcirc* 2002; 26: 99-106.
- Kim A1, Dadgostar H, Holland GN, Wenby R, Yu F, Terry BG, et al. Hemorheologic abnormalities with HIV infection: altered erythrocyte aggregation and deformability. *Invest Ophthalmol Vis Sci* 2006; 47: 3927-3932.
- Wilkins EG, Fraser I, Barnes, A, Khow S, Hamour A. Plasma viscosity in HIV infection. *Int Cont AIDs* 1992; 8: 143.