Original Research Article

P53 PROTEIN LEVEL AND EVIDENCE OF ONGOING COAGULATION AMONG HIV-INFECTED PERSONS ACCESSING TREATMENT AT UNIVERSITY OF CALABAR TEACHING HOSPITAL NIGERIA

ABSTRACT

Aims: To assess tumour suppressing activity and ongoing coagulation among persons living with HIV infection in Calabar, Nigeria.

Study Design: Case-control study

Place and Duration of Study: university of Calabar Teaching Hospital Calabar, Nigeria, between April 2018 and November 2018.

Methods: Ninety persons living with HIV infection who were attending clinics at University of Calabar Teaching Hospital were enrolled with ninety age and sex-matched HIV sero-negative individuals who served as control subjects. Blood specimen was collected from each participant for analyses of CD4 cell and full blood counts by automation, serum was used for the assays of P53 protein and D-dimer levels using enzyme-linked immunosorbent assay test kits.

Result: The CD4 cell count and P53 protein level reduced while D-dimer level increased in HIV infection. Platelet count also reduced while platelet distribution width increased with the condition. While CD4 cell count improved with Highly Active Antiretroviral Therapy administration, D-dimer level, mean platelet volume and platelet distribution width reduced.

Conclusion: This study observed reduced tumour suppression and increased coagulation activities alongside immunosuppression in HIV infection.

Keywords: Coagulation, HIV infection, immunosuppression.

1. INTRODUCTION

The health challenges associated with HIV infection in Africa are often compounded by issues that border on ignorance and poverty. It has been previously observed that in Calabar, screening for HIV infection is mainly occasioned by conventional antenatal care and prospective blood

Comment [I1]: Insert between infection and in "among person for care"

Comment [I2]: Teaching Hospital, Nigeria.

Comment [I3]: University

donation [1]. Unfortunately, the conventional antenatal care is yet to be fully accessed with the result that some infected pregnant women are not detected on time and the risk of vertical transmission continues to be a challenge [2]. Thus, late presentation to hospital remains a militating factor to early intervention [3]. Among the infected persons in our local population, widespread derangement in biomarkers and morbidity indicators that mirror poor health status prevail [4]. However, disease progression from HIV infection to acquired immune deficiency syndrome (AIDS) depends on proper management which in turn relies on timely detection of morbidity indicators and subsequent intervention [4,5].

An important aspect of the viral invasion of host immunity in HIV infection is the depletion of the T-helper CD4 cell population. In most resource-poor settings where viral load cannot be ascertained, CD4 cell count remains the biomarker for severity of infection and its subsequent progression to AIDS. Its degree of depletion is considered in the assessment of the severity of immunosuppression. While there is much focus on immunosuppression, the attendant morbidities of HIV infection are rarely investigated locally, thus limiting the scope of management and care offered to infected persons. Coagulation disturbances and cancer have been identified as factors for increased mortality among people living with HIV infection. Impaired immunity and the development of these other morbidities are thought to reflect an unending cycle that eventually progresses HIV infection to AIDS [6, 7, 8, 9,10]. In Nigeria, particularly Calabar, not much is known about the nature of the hemostatic disturbance seen in HIV infection. There is also paucity of information on levels of cancer biomarkers among infected subjects. This study was carried out with a view to assessing tumour suppressing activity and ongoing coagulation among HIV-infected subjects.

2. MATERIAL AND METHODS

Ninety persons living with HIV infection who were attending clinics at University of Calabar Teaching Hospital were enrolled with ninety age and sex-matched HIV sero-negative individuals who served as control subjects. The enrollment of persons living with HIV infection took into consideration certain sub-groups on the basis of commencement of highly active antiretroviral therapy (HAART). Thirty persons were newly diagnosed and were yet to embark on HAART. The remaining 60 were already undergoing treatment. Blood specimen was collected from each participant for analyses of CD4 cell and full blood counts by automation, serum was used for the assays of P53 protein and D-dimer levels using enzyme-linked immunosorbent assay test kits. Data analysis was done using SPSS version 22.0. Student t-test was used to compare means between test and control subjects. One-way analysis of variance was used to compare means across the HAART-naïve and two other groups on different HAART protocols. Pearson's correlation was used to analyze relationships. Statistical significance was drawn at a $p \leq 0.05$.

Comment [I4]: Please, which sampling technic was used from what sampling frame? More importantly, who was the sample size used for the study determined.

3. RESULTS AND DISCUSSION

3.1 Results

Persons living with HIV infection who participated in this study were adults from eighteen years and above. The age group with the highest number of participants was 36-45yeaars which featured 34.4% (31 out of 90) of all the persons. This was followed by age group 26-35years which had 31.1% (28 out of 90) of the subjects. The least number of participants, 7.8% (7out of 90) came from the group above 55years of age. More females 63.3% (57 out of 90) than males 36.7% (33 out of 90) were observed accessing medical care at the study center. In addition, more than half of these persons were married 60% (54 out of 90) at the time of the study. A third of the persons living with HIV infection were enrolled from those newly diagnosed. The remaining 60 were already undergoing treatment. Two HAART protocols were observed among subjects who

were being treated; Tenofovir+Lamivudine+Efavirenz (TLE) and Lamivudine+Zidovudine+Nevirapine (LZN). Subjects on TLE were 48.3% (29 out 60), while those on LZN were 51.7% (31 out of 60) (Table 1).

TABLE 1. Demographic parameters of studied subjects

Parameter	**HIV-infected persons n=90 (100%)**	**Control subjects n=90 (100%)**
Age (years)		
≤25	10 (11.1)	10 (11.1)
26-35	28 (31.1)	29 (32.2)
36-45	31 (34.4)	31 (34.4)
46-55	14 (15.6)	15 (16.7)
>55	7 (7.8)	5(5.6)
Gender		
Females	57 (63.3)	55 (61.1)
Males	33 (36.7)	35 (38.9)
Marital Status		
Single	29 (32.2)	36 (40.0)
Married	54 (60.0)	50 (55.5)
Widowed	7 (7.8)	4 (4.5)
HAART Initiation		
Treatment-Naïve	30 (33.3)	
Treatment on course	60 (66.7)	
HAART Protocol		
TLE	29 (48.3)	
LZN	31 (51.7)	

The CD4 cell count and P53 protein level were found to be reduced while D-dimer level increased in HIV infection. The platelet parameters considered in this study were platelet count, mean platelet volume (MPV) and platelet distribution width (PDW). Platelet count was observed to be reduced while platelet distribution width (PDW) increased with the condition (Table 2).

TABLE 2. CD4 cell count, P53 level, D-dimer level and Platelet parameters of HIV-infected persons and control subjects

Parameter	Control Subjects n=90	HIV-Infected Subjects n=90	p-Value
CD4 (cells/ml)	868.78±221.03	509.29±311.15	0.000
P53 (ng/l)	1816.87±575.33	1587.37±529.01	0.006
D-Dimer (pg/ml)	2816.33±696.38	4752.13±515.32	0.000
Platelet count (x 10^9/l)	255.26±64.31	215.99±59.98	0.000
MPV (fl)	9.13±0.86	9.21±0.95	0.550
PDW (%)	14.84±0.35	18.89±9.11	0.000

Subjects on highly active antiretroviral therapy (HAART) were either taking Tenofovir+Lamivudine+Efavirenz (TLE) or Lamivudine+Zidovudine+Nevirapine (LZN). Both drug combinations impacted similarly on the measured parameters. While CD4 cell count improved with HAART administration, D-dimer level, mean platelet volume (MPV) and PDW reduced (Table 3).

TABLE 3 Impact of routine HAART protocols on the measured parameters

Parameter	HAART-Naïve n=30	HAART (TLE) n=29	HAART (LZN) n=31	p-Value
CD4 (cells/ml)	377.63±191.18*	634.93±368.42	519.16±304.15	0.005
P53 (ng/l)	1653.20±555.32	1682.07±414.78	1435.06±578.43	0.138
D-Dimer (pg/ml)	4966.23±518.45*	4695.21±435.10	4598.19±526.79	0.014
Platelet count (x 10^9/l)	217.23±65.93	220.31±57.05	210.74±58.12	0.822
MPV (fl)	9.79±0.99*	8.81±0.81	9.02±0.78	0.000
PDW (%)	26.98±12.36*	14.89±0.56	14.80±0.50	0.000

Key: * =HAART-Naïve significantly different from both HAART (TLE) and HAART (LZN)

3.2 Discussion

Although HIV infection affects all ages, the current study enrolled persons from eighteen years of age and above mainly for the ease of obtaining consent. The age group with the highest number of participants was 36-45yeaars which featured 34.4% persons. This was followed closely by age group 26-35years which had 31.1% subjects. Altogether, the age group between 26-45 years constituted 65.5%. This frequency pattern for age, combined with that for gender (63.3% female participation) as well as that for marital status (60% of married persons) reveals a significant pattern. It implies that among adults living with HIV infection, women of child-bearing age constitute the highest group receiving medical attention for HIV infection. This trend has implications for the control of HIV infection in this locality as the risk of mother to child transmission could be better managed within conventional health facilities.

This study observed alongside a lower value of CD4 cell count, reduced serum p53 protein level. In the progression of HIV infection to AIDS, both decline in immunity and the development of cancer are considered important morbidity and mortality factors [6,8,9,10,11]. In resource-poor settings, cancer screening among HIV-infected persons is yet to commence despite the need to

monitor this aspect of health for infected persons [12]. The p53 gene and its protein play a significant role in the immunosuppression of cancer and is also kwon to mediate against the replication of the human immunodeficiency virus, thus serving as a host-restriction factor. It is therefore thought that the silencing of the p53 pathway promotes both viral replication and disease progression in HIV infection [13,14]. The two HAART protocols in use at the health facility were observed to improve the CD4 cell count but showed no significant variation for the serum p53 protein. There may be need to go beyond this stage of treatment if tumour immunosuppression is to be addressed. This could impact on disease progression from HIV infection to AIDS in Africa.

In addition to the reduced serum p53 protein, the studied population showed evidence of activated coagulation as observed in the lower platelet count but higher PDW and D-Dimer values. Although the finding of lower platelet count could arise from insufficient production as well as increased consumption, the observation of higher PDW value suggests the later. The PDW represents the variability in platelet size and is thought to be an important marker of platelet activation [7, 15,16,17,18,19] More importantly, D-dimer is the degradation product of fibrinogen and fibrin during fibrinolysis. Although there are various fibrin-degradation products that result from plasmin-mediated breakdown, D-dimers is considered a specific marker for fibrinolysis in that only fragments originating from fibrin polymers that had undergone factor XIII mediated cross-linking retain an intact covalent bond between two adjacent D domains; hence the term D-dimers. It therefore reflects ongoing activation of the hemostatic system and more specifically represent breakdown products of cross-linked fibrin clot formation [20,21,22].

Comment [I5]: known

Comment [I6]: Statement over specific, noting limitation of hospital-based research and the procedure for selecting sample not clear in this study.

4. CONCLUSION

This study concludes that there is reduced tumour suppression and increased coagulation activities alongside immunosuppression in HIV infection. The D-dimer, MPV and PDW mean values also varied across the HAART groups in relation to the HAART-Naïve group. The drugs impacted positively on the coagulation parameters studied, thus suggesting a better hemostatic state among persons living with HIV infection who are on HAART compared to those yet to commence HAART.

ETHICS APPROVAL AND CONSENT

Ethical approval was obtained from University of Calabar Teaching Hospital Health Research Ethics Committee, while written informed consent was obtained from each participant.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1 Akwiwu EC, Akpotuzor JO. Determinants of voluntary uptake of HIV counseling and testing among infected persons in Calabar, Nigerian. *Contemporary Journal of Inter-disciplinary Studies* 2018; 7(4): 48-54.

2 Ugochi VE, Akwiwu EC**,** Akpotuzor JO. Risk factors associated with HIV transmission and infection among persons aged 0-17 years in Calabar Metropolis of Nigeria. *Journal of Medical and Dental Science Research* 2018; 5 (5): 27-30.

3 Akwiwu EC, Akpotuzor JO, Okon JE, Egharevba O. Iron studies of HIV-infected subjects in Calabar- A Nigerian Perspective. *Journal of Medical Science and Clinical Research* 2017; 3(5): 19572-19577.

4 Ekanem BP, Akwiwu EC, Akpotuzor JO. Derangement in some antioxidants among HIV-infected persons in Calabar, Nigeria. *Journal of Pharmacy and Biological Sciences* 2018; 13 (3); 60-63.

5 Piwoz EG, Prebel EA. HIV/AIDS and nutrition: a review of the Literature and recommendations for nutritional care and support in sub-saharan Africa. *Washington DC Academy for Educational Development online report 2000.*

6 Biggar RJ, Chaturvedi AK, Geodert JJ, Engels EA. AIDS-related cancer and severity of immunosuppression in persons with AIDS. *Journal of National Cancer Institute* 2007; 99(12): 962-972.

7 Vagdatli E, Gounari E, Lazaridou E, Katsibourilia E, Tsikopoulou F, Labrianou I. Platelet distribution width: a simple, practical and specific marker of activation of coagulation. *HippoKratia*, 2010; 14(1):28-32

8 Cummins NW, Badley AN. Mechanisms of HIV-associated lymphocyte apoptosis. *Cell Death Disease*, 2010; 1:e99.

9 Riedel DT, Mwangi EI, Fantry LE, Alexander C, Hossain MB, Pauza CD et al. High cancer-related morality in an urban, predominantly African American, HIV-infected population. *AIDS*, 2013; 27(1): 1109-1171.

10 Coghill A, Shiels MS, Suneja G, Engels EA. Elevated Cancer-specific Mortality among HIV-infected Persons in the US. *Journal of Clinical Oncology* 2015; 33(21):2376-83.

11 Pinzone MR, Fiorica F, Dirose M, Malaguanera L, Cacopardo B, Zanghi C et al. Non-AIDS-defining cancers among HIV-infected people. *European Review of Medical and Pharmacology Science*, 2012; 16(10): 1377-1388.

12 Yang J, Su S, Zhao H, Wang D, Wang J, Zhang F et al. Prevalence and mortality of cancer among HIV-infected in patients in Beijing, China. *British Medical Centre of Infectious Disease*, 2016; 16: 1416-1423.

13 Theoret MR, Cohen CJ, Nahvi AV, Ngo LT, Suri KB, Powell DJ Jr. et al. Relationship between p53 overexpression of cancers and recognition by anti-p53 T cell receptor-transduced T cells. *Human Genetic Therapy*, 2008; 19(11), 1219-1232.

14 Yoon CH, Kim SY, Byeon SE, Jeong Y, Lee J, Kim KP et al. P53-derived host restriction of HIV-1 replication by protein kinase R-mediated Tat phosphorylation and inactivation. *Journal of Virology*, 2015; 89(8): 4262-4280

15 De Luca G, Venegoni L, Iorio S, Secco GG, Cassetti E, Verdoia M et al. (Novara Atherosclerosis Study Group). Platelet distribution width and the extent of coronary artery disease: results from a large prospective study. Platelets, 2010; 21(7):508-514.

16 Farias MG, Schunck EG, Dal Bó S, de Castro SM. Definition of reference ranges for the platelet distribution width (PDW): A local need. *Clinical Chemistry and Laboratory Medicine*,2010; 48(2):255-257.

17 Harsha MM, Chaithra SP. Thrombocytopenia in HIV infected patients and its correlation with clinical and immunological status. *Journal of Evolution of Medical and Dental Sciences*, 2013; 2 (27) 5035-5041.

18 Aydogan A, Akkucuk S, Arica S, Motor S, Karakus A, Ozkan OV. et al. The Analysis of Mean Platelet Volume and Platelet Distribution Width Levels in Appendicitis. *Indian Journal of Surgery:* 2015; **77**(2):495-500.

19 Osime EO, Oresanja OO, Okwara BU. Packed cell volume Platelet count, PT, PTTK and Fibrinogen concentration of HIV positive patients on antiretroviral drugs. *Pakistan Journal of Medical Sciences*, 2015; 31(6): 1533–1536.

20 Gaffney PJ. Fibrin degradation products; A review of structures found in vitro and in vivo. *Annals of New York Academy of Science*, 2001; 936:594-610

21 Borges AH., O'Connor JL, Phillips AN, Baker JV, Vjecha MJ, Losso MH. et al. Factors associated with D-dimer levels in HIV-infected individuals. *PLOS ONE*, 2014; 13: 9-13.

22 Riley RS. Widely used types and clinical applications of D-Dimer assay. *Laboratory Medicine*, 2016; 47(2):90-102.