

**REDUCED 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 and accessing care in University of Calabar Teaching Hospital, 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. 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. Statistical significance was drawn at a $p \leq 0.05$.

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, tumour suppression.

1. INTRODUCTION

33 The health challenges associated with HIV infection in Africa are often compounded by issues
34 that border on ignorance and poverty. It has been previously observed that in Calabar, screening
35 for HIV infection is mainly occasioned by conventional antenatal care and prospective blood
36 donation [1]. Unfortunately, the conventional antenatal care is yet to be fully accessed with the
37 result that some infected pregnant women are not detected on time and the risk of vertical
38 transmission continues to be a challenge [2]. Thus, late presentation to hospital remains a
39 militating factor to early intervention [3]. Among the infected persons in our local population,
40 widespread derangement in biomarkers and morbidity indicators that mirror poor health status
41 prevail [4]. However, disease progression from HIV infection to acquired immune deficiency
42 syndrome (AIDS) depends on proper management which in turn relies on timely detection of
43 morbidity indicators and subsequent intervention [4,5].

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

56 infected subjects. This study was carried out with a view to assessing tumour suppressing
57 activity (using serum p53 protein as a marker) and ongoing coagulation (using d-dimer in addition
58 to platelet parameters) among HIV-infected subjects.

59 2. MATERIAL AND METHODS

60 The prevalence of HIV infection in Cross River State, Nigeria was 4.4% as reported by National
61 Action Committee on AIDS in 2018. Although the sample size derived from the mentioned
62 prevalence is 64.6, it was extended to 90 because the study required comparable numbers from
63 three sub-groups (persons who were newly diagnosed and were yet commence treatment and
64 those who were being treated with either Tenofovir+Lamivudine+Efavirenz (TLE)
65 or Lamivudine+Zidovudine+Nevirapine (LZN). The study adopted mixed sampling method;
66 convenient sampling technique since the enrolled subjects were accessing care at the hospital and
67 a stratified approach to obtain comparable numbers from the sub-groups. Ninety persons living
68 with HIV infection who were attending clinics at University of Calabar Teaching Hospital were
69 enrolled with ninety age and sex-matched HIV sero-negative individuals who served as control
70 subjects. The enrollment of persons living with HIV infection took into consideration certain
71 sub-groups on the basis of commencement of highly active antiretroviral therapy (HAART).
72 Thirty persons were newly diagnosed and were yet to embark on HAART. The remaining 60
73 were already undergoing treatment. Blood specimen was collected from each participant for
74 analyses of CD4 cell and full blood counts by automation, serum was used for the assays of P53
75 protein and D-dimer levels using enzyme-linked immunosorbent assay test kits. Data analysis
76 was done using SPSS version 22.0. Student t-test was used to compare means between test and
77 control subjects. One-way analysis of variance was used to compare means across the HAART-

78 naïve and two other groups on different HAART protocols. Statistical significance was drawn at
79 a $p \leq 0.05$.

80 3. RESULTS AND DISCUSSION

81 3.1 Results

82 Persons living with HIV infection who participated in this study were adults from eighteen years
83 and above. The age group with the highest number of participants was 36-45years which
84 featured 34.4% (31 out of 90) of all the persons. This was followed by age group 26-35years
85 which had 31.1% (28 out of 90) of the subjects. The least number of participants, 7.8% (7out of
86 90) came from the group above 55years of age. More females 63.3% (57 out of 90) than males
87 36.7% (33 out of 90) were observed accessing medical care at the study center. In addition, more
88 than half of these persons were married 60% (54 out of 90) at the time of the study. A third of the
89 persons living with HIV infection were enrolled from those newly diagnosed. The remaining 60
90 were already undergoing treatment. Two HAART protocols were observed among subjects who
91 were being treated; Tenofovir+Lamivudine+Efavirenz (TLE) and
92 Lamivudine+Zidovudine+Nevirapine (LZN). Subjects on TLE were 48.3% (29 out 60), while
93 those on LZN were 51.7% (31 out of 60) (Table 1).

94 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)	

95
 96 The CD4 cell count and P53 protein level were found to be reduced while D-dimer level
 97 increased in HIV infection. The platelet parameters considered in this study were platelet count,
 98 mean platelet volume (MPV) and platelet distribution width (PDW). Platelet count was observed
 99 to be reduced while platelet distribution width (PDW) increased with the condition (Table 2).

100
 101 TABLE 2. CD4 cell count, P53 level, D-dimer level and Platelet parameters of HIV-infected
 102 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

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

110 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

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

112 **3.2 Discussion**

113 Although HIV infection affects all ages, the current study enrolled persons from eighteen years
114 of age and above mainly for the ease of obtaining consent. The age group with the highest
115 number of participants was 36-45years which featured 34.4% persons. This was followed
116 closely by age group 26-35years which had 31.1% subjects. Altogether, the age group between
117 26-45 years constituted 65.5%. This frequency pattern for age, combined with that for gender
118 (63.3% female participation) as well as that for marital status (60% of married persons) reveals a
119 significant pattern. It implies that among adults living with HIV infection, women of child-
120 bearing age constitute the highest group receiving medical attention for HIV infection. This trend
121 has implications for the control of HIV infection in this locality as the risk of mother to child
122 transmission could be better managed within conventional health facilities.

123 This study observed alongside a lower value of CD4 cell count, reduced serum p53 protein level.
124 In the progression of HIV infection to AIDS, both decline in immunity and the development of
125 cancer are considered important morbidity and mortality factors [6,8,9,10,11]. In resource-poor
126 settings, cancer screening among HIV-infected persons is yet to commence despite the need to
127 monitor this aspect of health for infected persons [12]. The p53 gene and its protein play a
128 significant role in the immunosuppression of cancer and is also known to mediate against the
129 replication of the human immunodeficiency virus, thus serving as a host-restriction factor. It is
130 therefore thought that the silencing of the p53 pathway promotes both viral replication and
131 disease progression in HIV infection [13,14]. The two HAART protocols in use at the health
132 facility were observed to improve the CD4 cell count but showed no significant variation for the
133 serum p53 protein. There may be need to go beyond this stage of treatment if tumour
134 immunosuppression is to be addressed. This could impact on disease progression from HIV
135 infection to AIDS.

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

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151 **4. CONCLUSION**

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

158 **ETHICS APPROVAL AND CONSENT**

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

161 **COMPETING INTERESTS**

162 Authors have declared that no competing interests exist.

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UNDER PEER REVIEW