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Role of apoptotic protease activating factor-1 in CD4+ depletion during HIV progression

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ABSTRACT

Objective: This study investigates the role of Apoptotic Protease Activating Factor-1 (APAF-1) in CD4+ cell depletion among human immunodeficiency virus (HIV) patients.

Materials and Methods: This is a cross-sectional study in which 105 participants were enrolled, including 60 confirmed HIV-positive patients and 45 HIV-negative controls. HIV-positive patients were further divided based on CD4+ cell counts: Group 1 (<200), Group 2 (200–499), and Group 3 (≥500). An enzyme-linked immunoassay was used to measure APAF-1 levels, and CD4+ T-cell counts were enumerated using a Cyflow counter. Independent student's *t*-test, Kruskal-Wallis, and Spearman's correlation were utilized as needed.

Results: Results showed significant reductions in lymphocytes, platelets, red blood cells, hemoglobin, albumin, and CD4+ cell values among HIV-infected individuals compared to controls. Conversely, APAF-1 and total protein levels were elevated in HIV-positive patients. Among HIV-positive groups, those with CD4+ cell counts <200 exhibited the highest median serum APAF-1 concentration. However, these differences were not statistically significant when compared with the other seropositive groups with CD4+ cell counts between 200 and 499 ($P = 0.6726$) and CD4+ cell counts of 500 or greater ($P = 0.4325$). The control group had the lowest median SAPAF-1 concentration, significantly different from HIV-positive groups. Positive correlations were observed between CD4+ counts and lymphocytes, hemoglobin, and hypoalbuminemia, while negative correlations were found between these parameters and APAF-1 levels.

Conclusion: APAF-1 is a host factor that potentially contributes to CD4+ cell depletion. Similarly, APAF-1, serum total protein, and albumin levels were found to be predictive of disease progression and could serve as valuable diagnostic biomarkers in the monitoring of HIV/AIDS.

Keywords: Apoptotic protease activating factor-1, CD4+ T cell, cytopenia, human immunodeficiency virus/acquired immune deficiency syndrome, hypoalbumin, progressor

Introduction

Infection with the human immunodeficiency virus type 1 (HIV-1) causes a gradual and irreversible depletion of CD4+ T-cells[1-3] CD4+ T-lymphocytes play a key role in immune responses; thus, a decrease in CD4+ T-cell counts may threaten the body's normal immunological functions. CD4+ T-cell depletion is frequently linked to the development of acquired immune deficiency syndrome (AIDS).[3,4] In other words, the quantity of CD4+ T-cells in blood circulation gives important information about the immune system.[5] The report shows that the presence of partial reverse transcripts in the abortively infected cells may imply that these immune cells have undergone apoptotic cell death as a result of innate DNA fragment sensing mediated by interferon-gamma-inducible protein 16.[6] The process of apoptosis has been extensively described as an established phenomenon in both plant and animal cells in the existing literature.[7] Even though apoptosis has been hypothesized as a critical mechanism of CD4+ T-cell depletion on numerous occasions, the process by which they die in HIV-infected hosts, particularly involving the intrinsic pathway, has so far received little attention.

Following the discovery of HIV about four decades ago, scientists postulated that CD4 T cell depletion plays a role in the pathological process of HIV-1 infection. Unfortunately, as investigations continued, multiple reports indicated that

apoptosis' function in HIV-1 pathogenesis is more convoluted than previously assumed, with one idea proposing that the Fas/Fas ligand (FasL) apoptotic pathway causes CD4 T cells to die progressively.[8] However, there has been no compelling evidence of the participation of Fas/FasL signaling in activation-induced cell death of T-lymphocytes among HIV-1-infected individuals, even though some studies have found evidence for Fas or FasL involvement.[9]

Contrastingly, according to a new study, tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), rather than FasL, appears to control the apoptosis of activated T-cells in HIV-1 patients.[10] Both FasL and TNF-associated apoptosis have been unequivocally recognized in the context of the extrinsic apoptotic pathway. However, new findings in the literature suggest that CD4 cell death occurs in the progression of HIV infection via both intrinsic and extrinsic pathways.[8] Unfortunately, there is a dearth of literature on the molecular process involving the intrinsic pathway to achieve T-cell death. Although several key molecules are involved in the induction of extrinsic TRAIL, FasL, and caspase,[10] and intrinsic B-cell lymphoma 2 signaling pathways have been identified,[11,12] the role of the apoptotic protease activating factor 1 (APAF-1) protein remains unknown.

Generally speaking, the APAF-1 gene, also known as the APAF1 gene, is responsible for the production of a cytoplasmic protein that causes apoptosis, albeit following an intrinsic pathway. Mechanistically, after binding cytochrome c and deoxyadenosine triphosphate (dATP), the APAF-1 protein has multiple copies of the WD-40 domain, as well as a caspase recruitment domain and an ATPase domain (NB-ARC) to form an oligomeric apoptosome. The apoptosome binds to and cleaves procaspase 9 after binding to cytochrome c and dATP, allowing the mature, active form to be released.[13] Even though the resultant caspase cascade that ends up killing the cell is believed to be triggered by activated caspase 9, the molecular basis underlying this reaction in HIV-1 pathogenesis is sparse in the literature.

Understanding the molecular mechanisms of CD4 cell depletion through effector molecules is believed to improve diagnostics and facilitate therapeutic strategies in the fight against HIV infection. The goal of this study was to investigate APAF-1 as a function of apoptosis in relation to CD4 cell depletion in HIV patients, as well as their correlations with hematopoietic function and plasma proteins.

Materials and Methods

Study area

This study was conducted at Sobi Specialist Hospital in Ilorin, Kwara State. Sobi Specialist Hospital is a secondary health-care facility founded in 1985 by the Kwara State Government in Nigeria's north-central region. Ilorin's main ethnic group is Yoruba, with minorities including Fulani, Hausa, Nupe, Bariba, Kanuri, and Igbo. According to the 2006 census, Ilorin has a population of 1,233,000 people.[14]

Study design

A cross-sectional analysis was used in this study.

Study population

- The study comprised a sample size of 105 participants, which was divided into two main groups: 60 patients with laboratory and clinically confirmed active HIV-positive and 45 HIV-negative individuals who served as the control group for comparison.
- The HIV-positive group was further categorized based on their CD4 cell counts, adhering to the WHO Case Definition guidelines for HIV progression. This categorization involved three subgroups:
  - CD4 <200: This subgroup included patients with severe CD4 cell depletion, indicative of advanced HIV progression (n = 14).
  - CD4 200–499: Patients with CD4 cell counts in this range were classified into this subgroup, representing a moderate level of immune depletion (n = 22).
  - CD4 ≥500: Individuals with CD4 counts ≥500 were placed in this subgroup, signifying relatively preserved immune function (n = 24).

The subjects were also evaluated over time to see if they were progressing or not, depending on their CD4 cell levels. This necessitated monitoring changes in CD4 counts and determining whether they were falling into "progressors" or improving as "non-progressors."

[Note: Progressors frequently show a decrease in CD4 cell counts over time, indicating HIV disease progression, whereas non-progressors have more stable or growing CD4 cell counts, showing better viral management or a less severe type of the disease].

The inclusion of an HIV-negative control group provided a valuable baseline for comparison, increasing the study's scientific rigor and the ability to make significant conclusions about the role of APAF-1 during HIV pathogenesis.

Inclusion criteria

HIV patients with confirmed status were included in the study.

Exclusion criteria

Patients who had a tuberculosis co-infection were excluded. Patients with hepatitis, diabetes, or a history of kidney disease were also excluded.

Sample size

Based on the most recent National Agency for the Control of AIDS (NACA) report,[15] the sample size was calculated using Fischer's expression, with the prevalence of HIV infection in Kwara State estimated at 1% based on the most recent NACA report. As a result, a minimum sample size of 27 was required, as shown below.

$$n = \frac{z^2 pq}{d^2}$$

n= the minimum sample size
z= the standard normal deviation = 1.96.
p= the prevalence rate of HIV in Kwara State is 1%[15]
q= 1.0−p = 1−0.018 = 0.982.
d= degree of accuracy desired, which is 5% (0.05).

$$n = \frac{(1.96)^2 \times 0.018 \times 0.982}{(0.05)^2} = 27$$

A total of 60 HIV seropositive patients were recruited for this study.

Ethical clearance

The Ethical Review Committee of the Kwara State Ministry of Health in Nigeria approved (Approval Number: MOH/KS/EU/777/306) this study. All subjects gave their informed consent, which was collected in compliance with human experimentation standards.

Sample collection and storage

Each participant had 10 mL of venous blood drawn, with 5 mL dispensed into a tri-potassium ethylene diamine tetra-acetic acid (K_3EDTA) vacutainer for CD4 count. The remaining 5 mL was emptied into plain bottles. For the serum APAF-1 (SAPAF-1) assay and other serum proteins, a blood sample from the plain bottle was spun, and serum was extracted and stored at −20°C.

Enumeration of blood CD4 T-cells

CD4 T-cells were enumerated using a Cyflow Cytometer, according to the manufacturer's instructions (Partec, Germany).

Procedure

Cyflow reagents and consumables were used according to the manufacturer's instructions. 20 µL of EDTA-anticoagulated blood specimens obtained from each study participant were dispensed into a partec test tube. 20 µL of CD4-phycoerythrin-conjugated monoclonal antibody supplied by partec was added to the tube containing the blood and then incubated for 15 min at room temperature in the dark. Following incubation, 800 µL of buffer, supplied by partec was added to the tube and gently vortex-mixed. The tube was then plugged into the Cyflow counter for automatic counting.

Percentage of CD4 T cells

The percentage of CD4 (%CD4) was calculated from the formula as follows:

$$\%CD4\,T\,Cell = \frac{CD4\,Count \times 100}{Absolute\,Lymphocyte\,Count}$$

Serum and urinary APAF-1 (UAPAF-1) determination

The concentrations of APAF-1 in the sera and urine of HIV-infected patients and control subjects were determined by the enzyme-linked immunoassay method.

Procedure

Fifty microliters (50 µL) of the standard were added to a standard well, and 40 µL of sample dilution was also added to the testing sample well, followed by the addition of 10 µl of the testing sample accordingly. A blank well was set along with the test. In the blank well, no addition of sample and HRP-conjugate reagent was done.

One hundred (100 µL) of HRP-conjugate was added to each well, except for the blank well. The plate was sealed with a plate membrane following incubation for 60 min at 37°C. 400 µL of washing buffer was added to every well for 30 s and then drained. It was repeated five times. The wells were dried by patting. 50 µL of chromogen A and 50 µL of chromogen B were also added to each well, and the plate was incubated for 15 min at 37°C. Then, 50 µL of stop solution was added to each well to stop the reaction while the blue color changed to yellow color. At this point, absorbance was read at 450 nm within 15 min of the addition of the stop solution.

Calculation of result

The concentration of APAF-1 was determined using the standard curve method. Each standard and sample were subtracted from the average zero standard optical density. A standard curve wa=1s constructed by plotting the absorbance for each standard on the y-axis against the concentration of APAF-1 on the x-axis, and the best-fit curve was drawn through the points on the graph.

Albumin estimation

Procedure

One thousand microliters (1000 µL) of diluted BCG reagent was pipetted into test tubes, 10 µL of serum and standard were added, and the content was properly mixed and incubated at 37°C for 10 min. The absorbance was read at 630 nm.

Calculation:

$$Albu\min(g/dL) = \frac{Absorbance\,of\ Sample}{Absorbance\,of\ Albu\min S\tan dard} \times Concentration\,of\ Albu\min Std\ (g/dL)$$

Total protein estimation (Biuret method)

Procedure

The Biuret reagent was prepared by dissolving 1.5 g of $CuSO_4.5H_2O$, 5 g of KI, and 6 g of Na-K-Ta in 0.2 M NaOH. 1000 µL of diluted Biuret reagent was pipetted into test tubes, and 20 µL of serum and standard were added, properly mixed, and incubated at 37°C for 30 min. The absorbance of the test was read against the standard after 10 min at 546 nm.

Calculation

The actual amount of total protein in g/L was determined using the formula below:

$$\Pr otein\ (g/dL) = \frac{Absorbance\,of\ Sample\times}{Absorbance\,of\ \ \Pr oteinS\tan dard} \frac{Concentration\,of\ \Pr otein\,Std\ (g/dL)}{}$$

Computation of total globulin

Total serum globulin concentration was determined by calculating the difference between total serum protein and serum albumin (SA), as reported elsewhere by.[16]

Total Serum Globulins (g/dL) = Tp−ALB

Determination of full blood count using an automated analyzer (as described by Sysmex, Germany)

Procedure

This method was previously employed.[17] The automatic voltage regulator, the uninterrupted power supply unit, and the power button on the Sysmex KX-21N hematology analyzer were activated. The "ready" status was allowed to appear, and a temporary hold-on period ensued to ensure the analyzer reached a "ready state." Subsequently, the subjects' sample information was input into the device. 5 mL of blood were collected in a K_3EDTA vacutainer and gently mixed. The vacutainer's stopper was opened to initiate the analysis. The tube was positioned at the sample probe, and the start switch was pressed to allow the device to aspirate the required volume. The tube was held against the sample probe until the buzzer sounded twice. Following each analysis, the device displayed and produced a hard copy of the report for documentation purposes. After printing, the machine resets to a ready state in preparation for the next sample.

Statistical analysis

Where appropriate, data were presented as a mean (standard error of mean [SEM]) and median (interquartile range). To determine predictor factors, the chi-square test was utilized. For categorical variables, proportions and percentages were calculated and compared using a 95% confidence interval. The independent sample student *t*-test was used to examine the mean. For divided subgroups with small sample sizes, the median values were evaluated using the Kruskal-Walli's test. To represent the variation trend between two continuous quantitative variables, Pearson's correlation was employed. The area under the curve (AUC) was determined as well as the receiver-operating characteristic (ROC) curves of the indices. Binary regression analysis was used to look at the predictive values of the selected potential parameters. $P < 0.05$ was considered significant.

Results

Table 1 shows the sociodemographic distribution of the study participants. The values for the mean (±SEM) for body mass index (BMI) and their co-infection status were also presented. The *P*-value was determined by the student's *t*-test, and $P < 0.05$ was considered significantly different.

In our analysis, the HIV+ group exhibited no statistically significant differences ($P = 0.284$) in terms of gender composition. Conversely, several hematological parameters, including lymphocyte counts (percentage) (LMP%), absolute lymphocyte counts, red blood cells ($\times10^{12}$/L), hemoglobin (HGB) (g/dL), mean corpuscular hemoglobin concentration (g/L), platelet counts (PLT) ($\times10^{9}$/L), CD4, and CD4%, demonstrated statistically significant reductions ($P < 0.05$) in HIV+ patients when compared to their HIV-negative counterparts.

Notably, the serum level of APAF-1 (pg/mL) was found to be elevated in HIV+ patients compared to the healthy population ($P < 0.05$), whereas the UAPAF-1 level (pg/24 h) exhibited a significant decrease ($P < 0.05$) when compared to the healthy group.

Furthermore, both the total protein (g/dL) and albumin (g/dL) levels in the serum of HIV+ patients were observed to be significantly lower ($P < 0.05$) than those in the HIV-negative population. These findings underscore the distinct biochemical and hematological alterations associated with HIV infection and emphasize the potential role of APAF-1 in the pathophysiology of HIV-related CD4+ depletion.

Tables 2a and b show SAPAF-1 concentrations in HIV seropositives stratified based on CD4+cell counts.

a. The group with CD4+ T cells <200 (<200 cell counts) had the highest median SAPAF-1 concentration; even so,

Table 1: Characteristics of studied subjects

Characteristics	Test (n=60)	Control (n=45)	P-value
Gender			
Male	23 (38.33)	21 (46.6)	0.284
Female	37 (61.67)	24 (53.33)	
Age range (years)			
<25	0 (0)	12 (26.67)	<0.001
26–41	35 (58.33)	18 (40.00)	
42–60	22 (36.67)	15 (33.33)	
>60	3 (5.00)	0.00	
Hematological indices			
White blood cell ($\times10^3/\mu L$)	4.660±0.22	4.903±0.20	0.561
LMP%	51.600±0.62	63.183±1.23	<0.001
LMP ABT	2.413±0.12	3.083±0.13	0.018
RBC (×1012/L)	4.127±0.08	4.880±0.19	<0.001
HGB (g/dL)	11.340±0.21	13.733±0.45	<0.001
MCV (fl)	89.807±1.09	80.600±0.84	<0.001
MCH (pg)	28.08±0.65	28.80±0.74	0.601
MCHC (g/L)	307.350±7.12	427.200±37.00	<0.001
PLT ($\times10^9$/L)	228.667±19.18	428.550±22.6	<0.001
CD4	437.500±38	931.467±44	<0.001
CD4%	14.700±1.48	39.467±2.66	<0.001
Serum APAF-1(pg/mL)	1233.517±53.54	1125.867±164.65	0.042
Urinary APAF-1 (pg/24 h)	789.500±9.49	1206.867±23.87	<0.001
T.P (g/dL)	7.342±0.11	6.740±0.24	0.026
ALB (g/dL)	4.353±0.14	5.013±0.12	0.009
Globulin (g/dL)	2.387±0.29	2.313±0.17	0.683
BMI (kg/m^2)	19.50±0.32	23.12±0.23	0.001
HBsAg	-ve	-ve	NA
Anti-HCV	-ve	-ve	NA
TB	-ve	-ve	NA

LMP%: Lymphocyte counts (percentage), LMP ABT: Absolute lymphocyte counts, RBC: Red blood cells, HGB: Hemoglobin, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, PLT: Platelet counts, T.P: Total protein, ALB: Albumin, BMI: Body mass index (kg/m^2), HBsAg: Hepatitis B surface antigen, Anti-HCV: Hepatitis C antibody, TB: Tuberculosis, NA: Not applicable, -ve: negative, SEM: Standard error of mean

the differences were not significant when compared with other seropositive groups of CD4+200–499 cell counts (P = 0.6726) and CD4+ ≥500 cell counts (P = 0.4325), respectively. The median concentration of SAPAF-1 was lowest in the control group and was statistically significant (P = 0.005, P < 0.001, and P < 0.001) when compared with seropositive groups with CD4 <200, CD4 200–499, and CD4 ≥500, respectively. Similarly, no significant differences in UAPAF-1 protein were found across HIV seropositive strata. However, the control group had the highest concentration of UAPAF-1 compared to the seropositive groups, which was statistically significant (P < 0.05).

b. The concentration of SAPAF-1 in HIV progressors was significantly higher (P < 0.05) compared to both non-progressors and the control group. Nevertheless, the concentrations of UAPAF-1 in progressors and non-progressors were significantly reduced (P < 0.05) compared to the control group.

Table 3 shows correlations between some selected parameters and CD4 T-cell counts and APAF-1 proteins.

In Table 3 below, the correlations between some selected parameters (white blood cell, lymphocyte [LYM], PLT, HGB, T.P., and albumin) and CD4 T cell count, SAPAF-1, and UAPAF-1 proteins of HIV-seropositive subjects are shown. The results showed positive correlations between LYM% and CD4 (r = 0.280; P = 0.030), LYM% and HGB (r = 0.231; P = 0.046), as well as albumin and CD cell counts (r = 0.618; 0.041), respectively. On the other hand, negative correlations were observed between CD4 and SAPAF-1 (r = −0.440, P<0.001), LYM and SAPAF-1 (r = −0.550; P = 0.034), LMP and SAPAF-1 (r = −0.422; P < 0.001), HGB and SAPAF-1 (r = −0.225; P = 0.048), and between albumin and SAPAF-1 (r = −0.823; P = 0.029), respectively.

Figure 1 depicts an ROC curve that tests the use of variables from the progressor and non-progressor HIV-positive groups as potential screening or diagnostic tools. Globulin has at least one link between the positive and negative actual state groups. Total protein and SAPAF had the highest AUCs (0.740 and 0.640, respectively) and high predictive values, with an overall area under the curve of 0.5. The highest sensitivity was recorded for the subjects' BMI at AUC (0.473), though this was not statistically significant [Figure 1].

The smallest cutoff value is the minimum observed test value minus one, and the largest cutoff value is the maximum observed test value plus one. All of the other cutoff values are the averages of two consecutively ordered observed test values.

Discussion

In this study, HIV infection was shown to be most common among those aged 26–41 years old, presumably because people in this age bracket are known to be sexually active. This is comparable to the findings of Volberding *et al*.,[18] who reported 30–44 years, and Essomba *et al*.,[19] who reported 35–45 years. As people of this age are in their most productive years and are meant to progress the nation's socio-economic growth, this could pose an effect of reducing the workforce and lowering productivity. Furthermore, according to male-to-female ratio, female participants made up approximately 62% of the total general population studied. This feminization supports the literature that women in this region are more likely than men to seek treatment in HIV clinics, and the biological and socio-cultural factors that characterize women's vulnerability to

Table 2: Comparisons of different variables among the studied groups

a) Comparison of SAPAF-1 and UAPAF-1 concentrations in different groups			
Group	**Staging; Case Definition (cell/μ)[14]**	**SAPAF-1 (pg/mL)**	**UAPAF-1 (pg/24 h)**
Group A (*n*=14) IQR	CD4 <200	1286[a] (1134.2–1437.8)	805[a] (771.7–838.3)
Group B (*n*=22) IQR	CD4 200–499	1196[b] (1130.7–1261.3)	790[b] (778.9–801.1)
Group C (*n*=24) IQR	CD4 ≥500	1172[c] (1107.3–1236.7)	781[c] (770.6–791.4)
Control (*n*=45) IQR	Not applicable	965.4 (938.3–992.5)	1207 (1183.1–1230.9)
P-value	Not applicable	<0.001*	<0.0001*
b) CD4 cell count, SAPAF-1 and UAPAF-1 levels in progressor, non-progressor and HIV-negative groups			
Group	**CD4 count (cell/μ)**	**SAPAF-1 (pg/mL)**	**UAPAF-1(pg/24 h)**
Progressor (*n*=20) IQR	147.7[a,c] (121–174.4)	1270[a,c] (1210–1330)	796.4[a] (772.4–820.4)
Non-progressor (*n*=29) IQR	645.7[b] (595.7–695.7)	1139[b] (1102–1176)	785.4[b] (775.1–795.7)
Control (*n*=45) IQR	931.5 (886.9–976.1)	965.4 (938.3–992.5)	1207 (1183.1–1230.9)
P-value	<0.001*	0.023*	0.643

Key: SAPAF-1 Serum apoptotic protease activating factor 1, UAPAF-1: Urinary apoptotic protease activating factor 1. The results were presented as median (interquartile range), with *P*-values computed using the Kruskal–Walli's test, with $P<0.05$ considered significantly different. [2a] a: significantly different, group A vs control; b: significantly different, group B*vs* controls; c: significantly different, group C vs controls, respectively [2b] a: Significantly different, progressor *vs* control groups; b: Significantly different, non-progressor control groups; c: Significant difference between progressor and non-progressor groups. *: Level of significance

Table 3: Correlations of selected parameters with CD4 T-cell counts and APAF-1 among the HIV-infected subjects

Parameters	CD4+ (cell/μ)		SAPAF-1 (pg/mL)	
	r	*P*	*r*	*P*
WBC	0.253	0.051	0.119	0.364
LYP%	0.880	0.030*	−0.550	0.034*
PLT	0.462	0.218	−0.263	0.832
HGB	0.531	0.046*	−0.225	0.048*
Albumin	0.618	0.041*	−0.823	0.029*
T.P	−0.202	0.121	0.055	0.677
CD4	1	–	−0.440	<0.001*
SAPAF-1	−0.440	<0.001*	1	-

*Correlation is significant at the 0.05 level (2-tailed); r: Correlation coefficient; WBC: White blood cell; LMP: Lymphocyte; SAPAF-1: Serum apoptotic protease activating factor 1, HGB: Hemoglobin. The *P*-values were determined by Spearman rho's non-parametric correlation matrix as appropriate

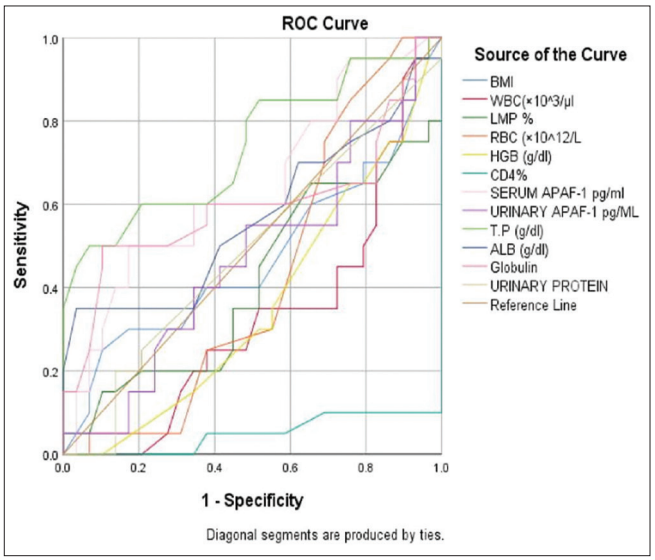

Figure 1: Receiver-operating characteristic curve illustrating the overall diagnostic performance of the assay

HIV infection in Sub-Saharan Africa may also play a role.[18] HIV infection suppresses the immune system by depleting the CD4 cell population via apoptosis; however, there are still controversies about the pathologic process to this day.

The majority of studies in the literature dwell on extrinsic pathways to unveil possible routes of mechanistic processes in HIV pathogenesis. Whether the intrinsic pathway is involved is still largely unknown. In the intrinsic pathway of apoptosis, the protein APAF-1 is the central component of the apoptosome, a multiprotein complex that activates procaspase-9 after cytochrome-c release from the mitochondria.[20] The role of apoptotic APAF-1 in the pathogenesis of HIV infection as a host factor for apoptosis has received less attention in the literature. The present study revealed that the SAPAF-1 concentrations across all strata of the HIV-infected individuals (considering their CD4+T cell levels) were significantly raised compared with the seronegative individuals. This showed there is a likelihood of aggravating apoptosis in HIV infection. Literature shows that APAF-1 is a crucial protein in the mitochondrial or intrinsic pathway of apoptosis, which oligomerizes in reaction to cytochrome c leakage and produces the apoptosome, a giant complex.[13] Thus, the process of CD4+ T cell death is assumed to have started with the recruitment of an apoptosome, which then activated procaspase-9, a mitochondrial pathway initiator caspase that leads to downstream caspase-3 processing.

Meanwhile, apoptosome formation and function can be influenced by a variety of cellular signals, either directly or indirectly. According to a previous study, HIV-1-infected patients have higher levels of inflammation than the general population.[21] In the current study, an increase in APAF-1 could be linked to a chronic inflammatory response in the HIV-infected group. In contexts of pathology, the mitochondria are at the heart of the pro-inflammatory response and play an important role in the immune

response to pathogenic infections. In essence, mitochondria are the key site for the generation of reactive oxygen species (ROS), or free radicals, which are required for infection resistance. Nevertheless, excessive and uncontrolled production of ROS could injure the cell, resulting in mitochondrial damage,[21] especially in chronic infections with persistent viral replication. During the HIV-1 replication cycle, infected CD4 T cells create cytosolic double-stranded DNA, which enhances mitochondrial ROS. Increased generation of ROS in infected CD4 T cells in HIV-1-infected patients is linked to CD4 T cell depletion.[22]

Furthermore, these intrinsic lethal stimuli, along with DNA damage and metabolic and ER stresses, among others, are thought to induce apoptotic molecules such as Bid, Bax, and Bak proteins. In consequence, these multiple death-inducing stimuli, Bid, Bax, and Bak, could induce the release of cytochrome C.[23] In addition, pro-inflammatory mediators known to trigger the extrinsic pathway of apoptosis could be linked with cytochrome c release in the mitochondria through the caspases 8 and 10/Bid/tBid/Bax/Bak route following the induction of death receptors (DR) (e.g., TRAILR and Fas). In this way, pro-inflammatory mediators such as interleukin (IL)-6, IL-1, and tumor necrosis factor may decrease albumin synthesis.[24] Meanwhile, the current study also found hypoalbuminemia among HIV-infected participants, which positively correlated with CD4 T cell counts. The association between the low albumin level and CD4 count in this study can be attributed to disease progression in the HIV-infected subjects. Low albumin levels have been linked to HIV progression.[25] Reduced SA levels, apart from reduced albumin synthesis,[24] may be associated with increased rates of albumin breakdown resulting from chronic inflammatory reactions that may orchestrate TNF-pathway signaling and potentially induce Bid-associated pro-apoptotic molecules to cause mitochondrial cytochrome c release, as previously stated. Even though hypoalbuminemia is associated with idiopathic chronic diarrhea, HIV enteropathy, and a decline in nutritional status,[24] all of which are common in HIV-positive people, albumin is a negative acute phase reactant, which means that its quantity decreases with inflammation. In other words, inflammation reduces albumin production largely due to increased levels of cytokines, particularly IL-1, IL-6, and tumor necrosis factor alpha (TNF-α).[26]

To support these findings, the current study also observed abnormal hematological indices–anemia, leukopenia, and thrombocytopenia-among the HIV-seropositive group, suggesting a potential inflammatory reaction in the pathologic process. According to the literature, cytopenia develops through different mechanisms. One popular explanation suggests that HIV infection increases the expression of pro-inflammatory cytokines such as TNF-α, TGF-β, and IL-1, resulting in myelosuppression and changes in the bone marrow microenvironment.[27] Although opportunistic infections, cancer, and HIV treatment have all been associated with the development of cytopenia,[28] given the study's case group selection process, blood cell destruction in this study could be more accurately attributed to pro-inflammatory immune-mediated destruction. Even though the concentration of UAPAF-1 decreased significantly in HIV-positive groups compared with healthy subjects, the decrease in UAPAF-1 in seropositives showed the likelihood of an increased metabolic rate in HIV subjects.

HIV seropositives were further stratified into progressors and non-progressors based on CD4+ T cell counts. This was done to rule out any underlying immune system problems and other genetic and virologic factors that may not have been ruled out during patient selection, as shown in Table 2b. According to the findings of this study, SAPAF-1 concentrations were higher during HIV progression compared to non-progression. This could be because non-progressors have a lower incidence of *in-vitro* spontaneous apoptosis of CD4+ T cells than progressors, which has been linked to lower levels of alpha interferon and DR5 in lymphoid tissue.[10] Even though there was no difference in UAPAF-1 concentration between the two groups, the process associated with elevated APAF-1 levels could be a hint that the protein is a product of inflammatory immune mediation. This finding demonstrates that HIV infection is a dynamic process with a variable degree of advancement in infected patients that differs depending on APAF-1 levels.

Furthermore, the ROC and AUC in this study [Figure 1] revealed that total protein and SAPAF-1 have the largest AUC (0.740) and (0.640), respectively, and are highly predictive, with an overall AUC of 0.5 and the highest sensitivity. The current study not only suggested that APAF-1 is a host factor in HIV pathogenesis, but it also suggested that it, like plasma proteins, could be useful for diagnostic and screening purposes.

Conclusion

According to the findings of this study, elevated APAF-1 levels in the blood are linked to lower CD4+ T cell counts as a result of chronic pro-inflammatory responses. Chronic immunological activation in HIV infection is connected to high levels of circulating pro-inflammatory cytokines and chemokines, which are linked to both intrinsic and extrinsic signaling pathways, according to the literature.[18] Given that APAF-1 is a downstream molecule of the intrinsic pathway, the possibility of inducing the TNF-apoptotic pathway through the Bid/Bax, Bak network still offers a huge potential for extrinsic pathway connection. Nonetheless, this research suggests that APAF-1 is a plausible host factor that contributes to the loss of CD4 cells in HIV patients. Furthermore, there is substantial evidence that APAF-1, serum total protein, and albumin could serve as diagnostic tools in the monitoring of HIV/AIDS disease progression.

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Patient's Consent Statement

The authors have obtained written consent from the patient (or legal guardian) to publish the manuscript, ensuring confidentiality and privacy.

Consent for Publication

All authors have reviewed and approved the final version of the manuscript for publication.

Availability of Data and Material

The authors commit to making the data and material related to the study available upon reasonable request. Additional details regarding data availability can be obtained by contacting the corresponding author.

Competing Interests

The authors declare that they have no competing interests, financial or otherwise, that could influence or be perceived to influence the research presented in the manuscript.

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Authors' Contributions

ALA: Designed and supervised the study. WOG: Carried out the laboratory work and collected the data. KAA and WOG: Analyzed the data. KAA, WOG, IM, OGA, and INA: Performed the background literature review. KAA: Drafted the manuscript. KAA and WOG: Revised the manuscript. All authors reviewed and approved the final version of the manuscript.

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