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Original Article

Association of Some Surrogate Inflammatory Markers and Non-invasive Measures of Liver Fibrosis among Patients with HIV Infection

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Abstract

Background and Aim: HIV is associated with inflammation and liver damage. Assay of inflammatory markers and liver biopsy are expensive for resource - poor countries to use routinely, necessitating the use of non - invasive markers. This study seeks to examine the association of some surrogate markers of inflammation with some non-invasive markers of fibrosis among patients with HIV infection in Benin City, Nigeria.

Methods: Venous blood was collected from all participants. The study was carried out in the University of Benin Teaching Hospital, Benin City, Nigeria. Surrogate inflammatory markers {such as neutrophil / lymphocyte ratio (NLR), platelet / lymphocyte ratio (PLR), mean platelet volume (MPV) and systemic immune - inflammatory index (SII)}, non - invasive markers of fibrosis {such as fibrosis index based on four factors (FIB-4), aspartate aminotransferase to platelet ratio index (APRI) and fibrosis - cirrhosis index (FCI)} and CD4 counts were evaluated in 125 patients with HIV infection {44 Highly Active Antiretroviral Therapy (HAART) - naïve and 81 on HAART)} and 48 non -HIV individuals using standard techniques.

Results: The prevalence of liver fibrosis among patients with HIV infection on HAART was 2.47%, 6.17% and 2.47% using APRI, FIB-4 and FCI respectively whereas only FIB-4 (6.82%) and FCI (2.27%) revealed liver fibrosis in HAART - naïve patients. NLR, SII and PLR were significantly higher in HAART - naïve patients than those on HAART. PLR was also significantly higher in HAART-naïve HIV patients compared to non-HIV patients ($p < 0.01$). FIB-4 was significantly higher in patients with HIV infection on HAART (1.54 ± 0.30) than in non-HIV individuals (0.54 ± 0.06) ($p<0.05$). PLR, MPV and CD4 count had a significant correlation with some of the markers of fibrosis in patients with HIV infection in the different study groups.

Conclusion: Liver fibrosis was detected more with FIB-4, and PLR was the only inflammatory marker that significantly correlated with all markers of liver damage among patients with HIV infection on HAART.

Keywords: Antiretroviral therapy; HIV; Inflammation; Liver fibrosis; Non-invasive markers; HAART.

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Introduction

Infection with the human immunodeficiency virus (HIV) is one of the most serious public health issues; it was reported to have affected an estimated 39 million people worldwide in 2022, with 37.5 million of them being adults (1). The introduction of highly active antiretroviral treatment (HAART) has systematically shifted the leading causes of morbidity and mortality among people living with HIV from opportunistic infections and AIDS - related neoplasms to non - AIDS related events, particularly cardiovascular and liver disorders especially in high - income countries (2, 3). Several studies have revealed the prevalence of liver involvement in persons living with HIV (PLWH) ranging from 4.4% to 14.5%, and

liver-related mortality accounting for up to 18% of deaths in some European real - life cohorts (4-6).

Liver fibrosis results from increased build-up of extracellular matrix proteins, including collagen that occurs in most forms of chronic liver disorders (7). There are several postulations on the mechanisms by which HIV result in liver damage. Amongst them are: HIV being a cytopathic virus may directly kill liver cells (Hepatocytes) as the HIV envelope glycoprotein, gp120, is thought to enter hepatic stellate cells (HSCs) via their coreceptors CCR5 and CXCR4, inducing death via collagen and tissue inhibition of metalloproteinase-1 activation, and subsequent generation of reactive oxygen species (8, 9). Also, HIV-induced $CD4^+$ T cell depletion has been demonstrated to limit the development of natural killer (NK) cells, which destroy activated HSCs and therefore modulate hepatic fibrosis. As a result, a profibrogenic milieu may be created by reduced NK activity produced by $CD4^+$ T cell depletion. HIV infection and antiretroviral therapy (ART), particularly older nucleoside reverse transcriptase inhibitors (NRTIs) and protease inhibitors (PIs), can cause mitochondrial damage (10-12). Intracellular lipid build-up, necrosis, and hepatotoxicity result from loss of mitochondrial function with impairment of mitochondrial fatty acid beta-oxidation and oxidative phosphorylation, may contribute to liver dysfunction in PLWH (10, 12).

During an infectious response, two major populations of T-lymphocytes play crucial roles: $CD8^+$ T cells are fundamental to directly kill infected cells, whereas $CD4^+$ T cells help $CD8^+$ T cells and sustain the maturation of highly specific antibodies produced by B-lymphocytes and therefore are essential elements for an effective immune response (13). Also, proinflammatory cytokines such as tumor necrosis factor, interferons and interleukin 1 along with eicosanoids and microRNAs form an interactive network during HIV infection. Therefore, in HIV infection the cross - regulation between proinflammatory mediators imparts strongly on infected cell death patterns which in turn affect inflammation (14). Anemia, lymphocytopenia, neutrophilia, monocytosis, monocytopenia, thrombocytopenia, and thrombocytosis are among the hematological diseases known to be associated with HIV (15). However, in resource limited settings like ours there is a lack of capacity to assess these immune - markers of inflammation routinely hence some surrogate markers of inflammation such as platelet - to - lymphocyte ratio (PLR), monocyte - to -lymphocyte ratio (MLR), and neutrophil - to -lymphocyte ratio (NLR) has all been discovered as possible markers of inflammation in a variety of illnesses, including malignancies and cardiovascular disease (16-19). Also, the monitoring of liver fibrosis can also be cumbersome and expensive because liver biopsy which has remained the gold - standard for diagnosis and staging of liver fibrosis has many limitations in its application in routine clinical practice (such limitations are need for experienced personnel, high cost, sampling errors, interobserver variability) and complications (bleeding, pain and rarely death) (20). Therefore, non - invasive evaluation of liver fibrosis using easy to do liver function test parameters like aspartate aminotransferase to platelet ratio index (APRI), fibrosis cirrhosis index (FCI) and Fibrosis-4 (FIB-4) using accurate and accessible methods is gaining acceptance around the world (21, 22). The goal of this study therefore, is to evaluate the association of some surrogate markers of inflammation and these non-invasive markers of fibrosis in patients with HIV infection attending clinic in the University of Benin Teaching Hospital in Benin City, Nigeria.

Methods

Study location and population

This study was carried out in the University of Benin Teaching Hospital (UBTH), Benin City, Nigeria. The hospital is a tertiary hospital with over 900 beds and has a referral status. The study was conducted using 125 patients with HIV infection attending PEPFAR Clinic in the University of Benin Teaching Hospital, Benin City, Edo State, Nigeria. Of the 125 patients, 81 of them were already undergoing treatment with the Highly Active Antiretroviral Therapy (HAART) while the remaining 44 were yet to commence the therapy and were designated HAART-naïve. The study population also consisted of 48 participants who were apparently and seronegative for HIV and served as controls. The controls were apparently healthy individuals without symptoms of any ailment. These individuals came for voluntary HIV testing in our Institution. Those that tested negative for HIV was approached discussed with and those that gave their consent were recruited for this study. The HIV testing in our setting followed the approved protocol from the Federal Ministry of Health. All subjects were asymptomatic. Written

informed consent was obtained from all participants prior to specimen collection.

The regimens for HAART are divided into first line, alternate first line and second line drugs. The first line and alternate first line HAART regimens are zidovudine, lamivudine and nevirapine, or combivir (consisting zidovudine, lamivudine) and liponavir/ritonavir, or combivir and efavirenz, or combivir and abacavir, or abacavir, lamivudine and nevirapine. The second line HAART regimen entails any of the following combinations: abacavir, lamivudine and liponavir/ritonavir, or zidovudine, lamivudine and liponavir/ritonavir, or stavudine, lamivudine and liponavir/ritonavir, or abacavir, lamivudine and aluvir or efavirenz, lamivudine and tenofovir, or efavirenz and ritonavir, or efavirenz, lamivudine, disoproxil fumarate. The Ethical Committee of Edo State Ministry of Health, Benin City, Nigeria, approved the protocol for this study.

Inclusion and exclusion criteria

Subjects used in this study included HIV positive patients with or without HAART therapy as subjects and apparently healthy individuals without HIV infection as controls. For the purpose of this study, the following groups of people were excluded:

1. Pregnant female patients since pregnancy can result in inflammation. Among the female participants recruited as mentioned above, Beta-hCG was performed to rule out pregnancy.
2. Individuals with other known co-morbidities like diabetes, hypertension, HBV and HCV.

Sample collection and processing

Six millilitre (6 ml) of blood sample was aseptically drawn from each of the participants by veni- puncture; 3ml of the blood sample was dispensed into Ethylene diamine tetra - acetic acid (EDTA) anticoagulated container and mixed properly to avoid clotting and the remaining 3ml was dispensed into plain container and allowed to clot.

Serum was separated from the clotted blood sample in the plain container and stored at -20^{o}C. This was used for liver function tests.

Full Blood Count Analysis

Full blood count (complete blood count) was determined with the EDTA blood sample using a hematology auto - analyzer - Sysmex K2IN (Sysmex Corporation, Kobe, Japan) by following the manufacturer's instructions.

Inflammatory Markers

Some basic inflammatory markers such as neutrophil/lymphocyte ratio (NLR), platelet /lymphocyte ratio (PLR), mean platelet volume (MPV), and systemic immune-inflammatory index (SII) were calculated from the obtained parameter of the full blood count as previously described (19). Briefly, NLR was calculated as the ratio of the neutrophils count to lymphocyte counts while PLR was calculated as the ratio of the platelet count to lymphocyte counts. The SII was defined as follows: SII= neutrophil × platelet / lymphocyte.

Liver Function Test

The serum samples obtained were used to determine the following biochemical parameters - albumin, bilirubin, alkaline phosphatase, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) using Selectra Pros chemistry auto analyzer (Vital Scientific Inc., Germany) following the manufacturer's instruction.

Non-invasive Markers of Liver Fibrosis

Non-invasive markers of liver fibrosis such as fibrosis index based on four factors (FIB-4), aspartate aminotransferase to platelet ratio index (APRI) and fibrosis-cirrhosis index (FCI) were calculated from the obtained liver function parameters and platelet values using the formula and cut-off values previously described by Kliemann *et al*. (22) as follows:

The APRI was calculated as:

$$APRI = \frac{(\text{AST/upper limit of normal}) \text{ x } 100}{\text{platelet count } (10^9/\text{L})}$$

APRI < 0.5, no or minimal fibrosis was assumed.

APRI > 1.5, patients were classified as having significant fibrosis

The FIB-4 score was based in the Sterling formula:

$$FIB-4 = \frac{\text{Age [years]} \times \text{AST } \left[\frac{\text{IU}}{\text{L}}\right]}{(\text{platelet count } [10^9/\text{L}] \times (\text{ALT1/2 [IU/L]}).}$$

FIB-4 < 1.45, no or minimal fibrosis was assumed.

FIB-4 > 3.25, patients were classified as having significant fibrosis.

The FCI was calculated as following:

$$FCI = \frac{(\text{alkaline phosphatase x bilirubin})}{[\text{albumin x platelet count } (10^9/\text{L})].}$$

FCI < 0.13, patients were classified as having no or minimal fibrosis;

FCI > 1.25, patients were considered as having cirrhosis.

Statistical Analysis

The non - parametric data obtained were analyzed using the Chi-square ($\chi 2$) test, while the parametric data were analyzed with student's t-test, ANOVA and correlation using the statistical software INSTAT® version 2.05 for Windows 7 (Graph Pad Software, La Jolla, California USA). Level of significance was set at $p < 0.05$.

Results

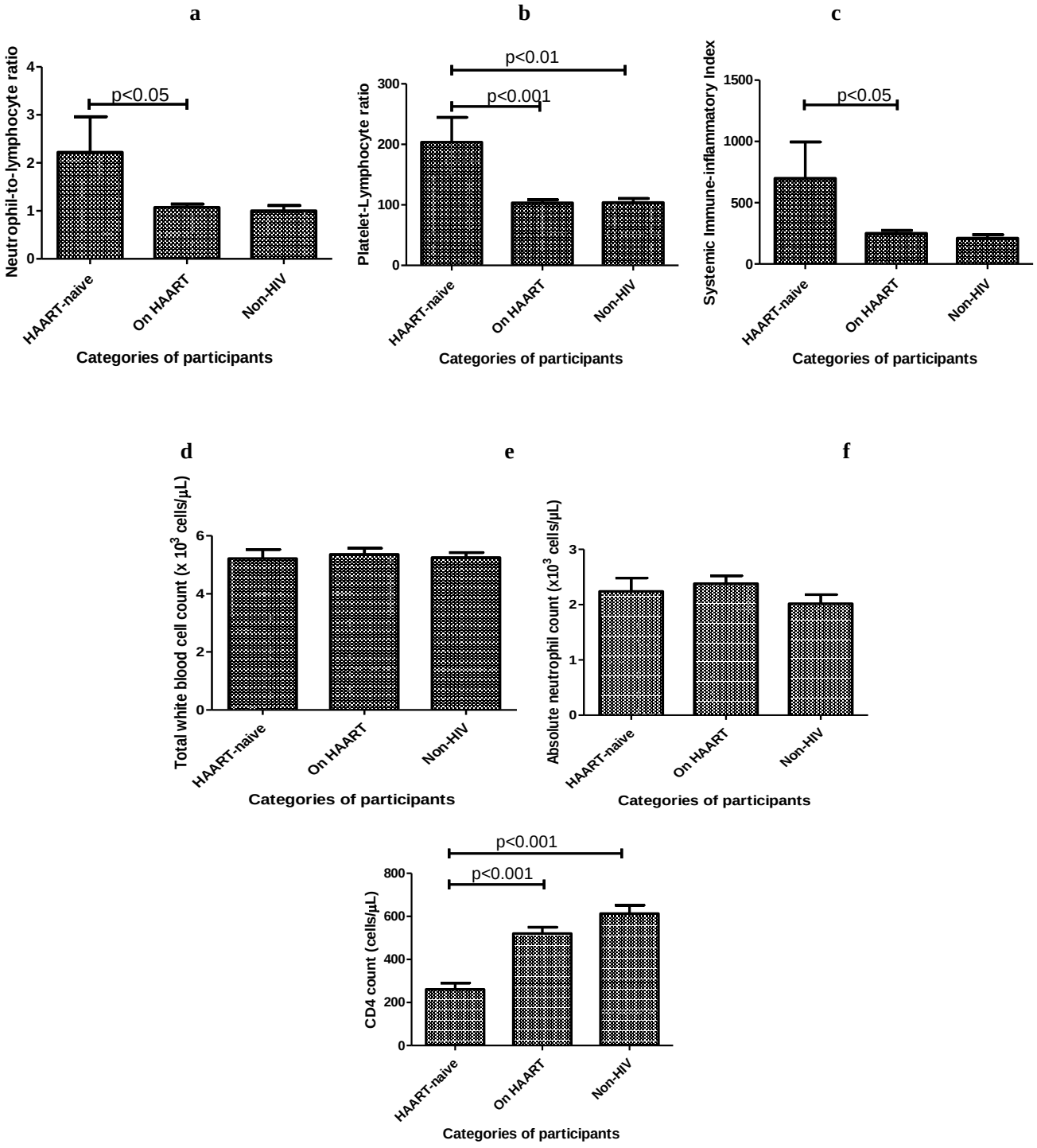
The study compared the level of some surrogate markers of inflammation with the various study groups and it reveals that there was no statistically significant difference in the absolute neutrophils and lymphocytes count amongst the study groups. However, NLR and PLR were significantly higher in HAART-naïve patients with HIV infection than those on HAART at $p< 0.05$ and $p < 0.01$ respectively (Fig. 1a, 1b). Also, PLR was significantly higher in HAART - naïve patients with HIV infection compared to non - HIV individuals ($p < 0.01$) (Fig. 1b). SII was significantly higher in HAART - naïve patients with HIV infection than those on HAART ($p < 0.001$) (Fig. 1c). CD4 count of HAART - naïve patients with HIV infection was significantly lower than the CD4 count of both patients with HIV infection on HAART and non - HIV individuals ($p < 0.001$) (Fig. 1d). Although the values of all three non - invasive markers of liver fibrosis were higher in patients with HIV infection irrespective of treatment status, only FIB-4 values reached statistical significance (HAART - naïve; $p < 0.001$, and on HAART; $p < 0.001$) in comparison with non-HIV subjects (Fig. 2a, 2b, 2c). A summary of these results is presented in table 1.

Table 1. Comparison of Some Blood Cell Parameters, Surrogate Markers of Inflammation and Non-invasive Markers of Liver Fibrosis among the Study Groups

Parameters	HAART - naïve (n=44)	On HAART (n=81)	Non - HIV (n=48)
Total White Cell Count ($x10^3$ cells/µL)	5.22 ± 0.31	5.36 ± 0.21	5.25 ± 0.17
Absolute neutrophil count ($x10^3$ cells/µL)	2.24 ± 0.24	2.38 ± 0.14	2.02 ± 0.16
Absolute lymphocyte count ($x10^3$ cells/µL)	2.04 ± 0.21	2.53 ± 0.15	2.25 ± 0.15
Platelets Count ($x10^3$ cells/µL)	242.66 ± 11.69	225.36 ± 8.99	235.98 ± 9.10
Neutrophil-to-Lymphocyte ratio (NLR)*	2.22 ± 0.74	1.07 ± 0.07	1.00 ± 0.11
Platelet-to-Lymphocyte ratio (PLR)*,†	203.72 ± 40.92	103.33 ± 5.35	103.96 ± 6.78
Mean platelet volume (fL)	10.31 ± 0.13	10.19 ± 0.14	10.45 ± 0.14
Systemic Immune-inflammatory Index (SII)*	699.70 ± 295.80	250.85 ± 23.35	210.35 ± 28.56
CD4 count (cells/µL)*,†	261.27 ± 29.02	520.16 ± 29.40	613.07 ± 38.53
APRI	0.44 ± 0.05	0.45 ± 0.09	0.27 ± 0.02
FIB-4†,ɣ	1.50 ± 0.16	1.54 ± 0.30	0.54 ± 0.06
FCI	0.16 ± 0.04	0.16 ± 0.04	0.12 ± 0.02

HAART: highly active antiretroviral therapy; **FIB-4:** fibrosis index based on four factors; **APRI:** aspartate aminotransferase to platelet ratio index; **FCI:** fibrosis - cirrhosis index.

Values are mean ± SEM; NLR - *HAART-naïve vs on HAART: $p < 0.05$; PLR - *HAART - naïve vs on HAART: $p<0.001$, † HAART-naïve vs Non-HIV: $p < 0.01$; SII - *HAART - naïve vs On HAART: $p < 0.05$; CD4 - *HAART- naïve vs On HAART: $p < 0.001$, †HAART - naïve vs Non - HIV: $p < 0.001$; FIB - 4 - HAART - naïve vs Non - HIV: $p < 0.001$, ɣOn HAART vs Non - HIV: $p < 0.001$.



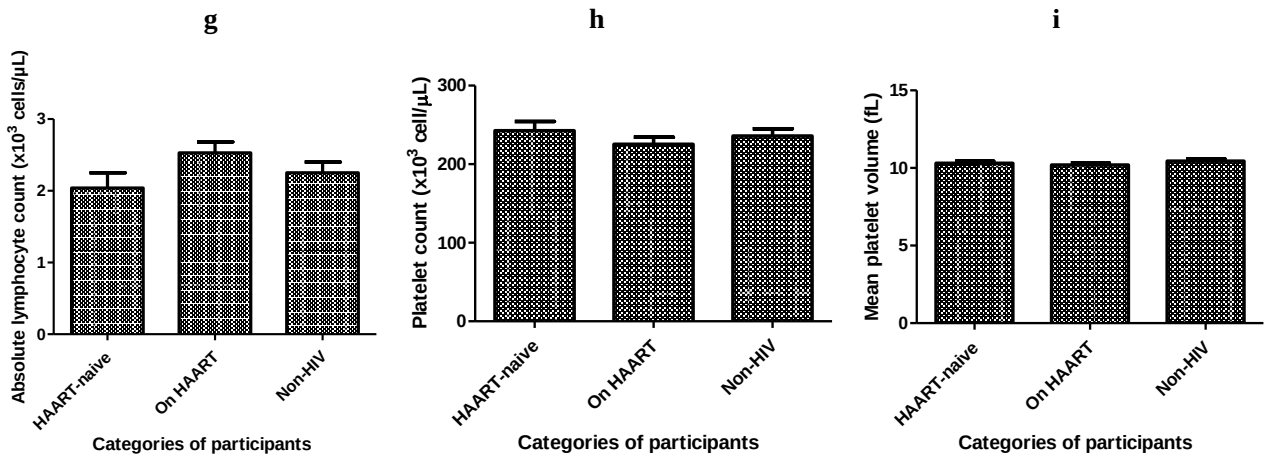


Figure 1. Comparison of some blood cell parameters and surrogate markers of inflammation among the study groups. **a:** Neutrophil - to - Lymphocyte ratio (NLR)* amongst study participants. **b:** Platelet - to - Lymphocyte ratio (NLR)* amongst study participants. **c:** Systemic Immune-inflammatory Index (SII)* amongst study participants. **d:** CD4 counts (cells/uL)* amongst study participants. **e:** Total white blood cell count (x103cell / uL) amongst study participants. **f:** Absolute neutrophil count (x103 cells / µL) amongst study participants. **g:** Absolute lymphocytes count (x103 cells / µL) amongst study participants. **h:** Platelet count (x103 cells / µL) amongst study participants. **i:** MPV levels amongst study participants.

HAART: highly active antiretroviral therapy; **FIB-4:** fibrosis index based on four factors; **APRI:** aspartate aminotransferase to platelet ratio index; **FCI:** fibrosis - cirrhosis index.

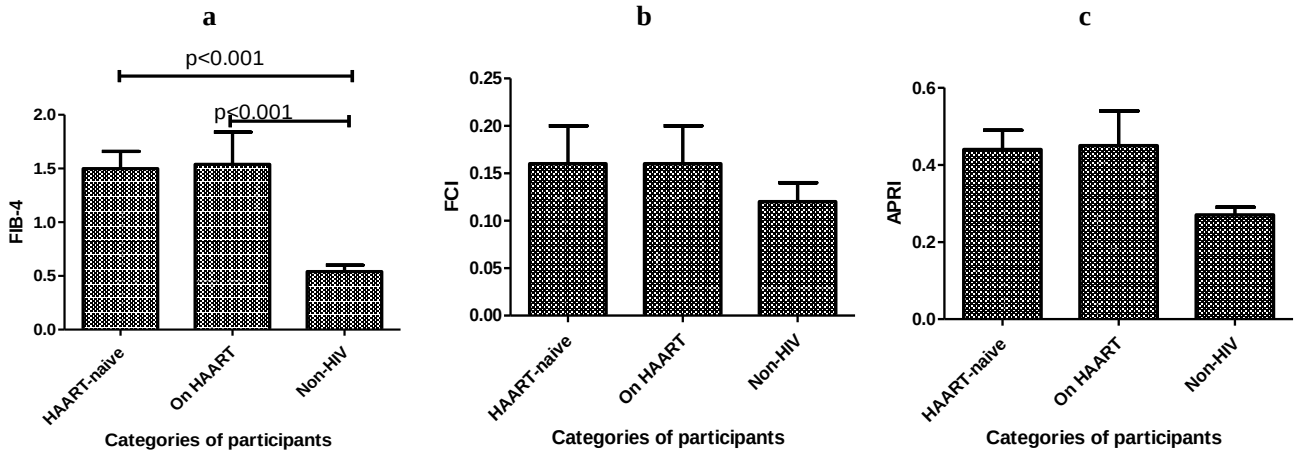


Figure 2. Comparison of Non-invasive markers of liver fibrosis among the study groups. **a:** fibrosis index based on four factors (FIB-4). **b:** fibrosis-cirrhosis index (FCI). **c:** aspartate aminotransferase to platelet ratio index (APRI).

NLR, PLR, SII, and CD4 correlated negatively with the non - invasive markers of liver fibrosis across study groups, with the exception of NLR and APRI in non - HIV individuals. In contrast, MPV had a positive correlation with them across study groups, with the exception of FIB-4 in the non - HIV group. Despite the fact that these associations were largely insignificant ($p > 0.05$), PLR was significant ($p< 0.05$) for all fibrosis indicators in both patients with HIV infection taking HAART and non - HIV participants (except FCI in non-HIV [$p > 0.05$]). In HAART - naive patients with HIV infection as well as non-HIV individuals, MPV significantly ($p < 0.05$) correlated with FCI as well as FIB-4. CD4 count only correlated significantly ($p < 0.05$) with FCI in non-HIV individuals, and SII only significantly ($p< 0.05$) correlated with FIB-4 in HIV patients on HAART (Table 2).

Table 2. Correlation of Non-invasive Markers of fibrosis with some surrogate markers of inflammation

	Correlation Coefficients (r)		
Inflammatory markers	**APRI**	**FIB-4**	**FCI**
HAART-naïve			
NLR	-0.0641	-0.0603	-0.0217
PLR	-0.1761	-0.2057	-0.0844
MPV	0.3123	0.3194*	0.3419*
SII	-0.0771	-0.0721	-0.0172
CD4	-0.1494	-0.1478	-0.1188
On HAART			
NLR	-0.1081	-0.1296	-0.1134
PLR	-0.3069*	-0.3755*	-0.3193*
MPV	0.0847	0.1161	0.0633
SII	-0.2023	-0.2371*	-0.1867
CD4	-0.0656	-0.0626	-0.0055
Non-HIV			
NLR	0.1262	-0.1134	-0.0003
PLR	-0.2983*	-0.3812*	-0.2640
MPV	0.2783	-0.0382	0.3701*
SII	-0.0487	-0.2110	-0.1579
CD4	-0.1998	-0.1389	-0.3024*

*Significant correlation ($p < 0.05$).

HAART: highly active antiretroviral therapy; **FIB-4:** fibrosis index based on four factors; **APRI:** aspartate aminotransferase to platelet ratio index; **FCI:** fibrosis - cirrhosis index.

The study also revealed an overall prevalence of 7.5% liver fibrosis using all of the non-invasive markers of liver fibrosis and there was a higher prevalence among HIV patients on HAART (11.1%) with none detected among the non - HIV group. There was no significant difference ($p = 0.5862$) in the use of any of the markers in the detection of fibrosis across the group of study participants (Table 3).

Table 3. Prevalence of liver fibrosis among study population

Subjects	APRI (%)	FIB-4 (%)	FCI (%)
HAART-naïve (n=44)	0 (0.00)	3 (6.82)	1 (2.27)
On HAART (n=81)	2 (2.47)	5 (6.17)	2 (2.47)
Non-HIV (n=48)	0 (0.00)*	0 (0.00)*	0 (0.00)*

P = 0.5862 *- not included for analysis

HAART: highly active antiretroviral therapy; **FIB-4:** fibrosis index based on four factors; **APRI:** aspartate aminotransferase to platelet ratio index; **FCI:** fibrosis - cirrhosis index.

Discussion

Human immunodeficiency virus (HIV) infection is associated with persistent proinflammatory responses in patients receiving antiretroviral therapy (ART) (23). Inflammation in HIV is linked to worsened outcomes, including death, the development of acquired immunodeficiency syndrome, and cirrhosis (especially in the presence of hepatitis C virus co-infection) (23, 24). In order to evaluate the relationship between some non-invasive inflammatory

markers and some surrogate fibrosis markers, this study was conducted.

Although haematological abnormalities are common complications of HIV infection, our study revealed that there was no statistically significant difference in the absolute neutrophils and lymphocytes count between patients who have HIV (on HAART and HAART naïve) and non-HIV (controls). A few reports have identified some of the alterations, such as low haemoglobin and platelets, as being reported in the early stages of HIV infection, while some authors have claimed that in the majority of cases of HIV infection, haematological abnormalities only appear in the middle or later stages of the infection (25, 26). This may be the reason behind our findings as none of our HIV participants were known to be beyond HIV clinical stage 1.

Significantly higher ($p < 0.05$) values of NLR, PLR and SII among HAART-naïve patients with HIV infectioncompared with their counterparts on HAART was observed in this study. HAART has been reported to reduce systemic inflammation and immune activation (27). This may explain the finding in this study. As the viral load decreases with the use of HAART medications (28), it is anticipated that the CD4 count will rise (29). This agrees with the finding in this study.

Blackard *et al* (30) had previously reported that there is a relationship between HIV infection and liver fibrosis, and most HAART drugs have been reported to cause liver fibrosis (31). This agrees with the finding in this study. Only FIB-4 among the markers of liver fibrosis showed higher values. This would suggest that FIB-4 is better capable of identifying liver fibrosis. In fact, FIB-4 is one of the recommended indicators of liver fibrosis in settings with limited resources (32).

PLR and fibrosis indicators were found to significantly correlate in this investigation, particularly in patients with HIV infection using HAART. Inflammation can lead to liver fibrosis (33). In this study, the inflammatory markers were higher in HAART-naïve patients with HIV infection than their counterparts on HAART. It is known that HAART damages the liver (34). It has been reported by some authors that HAART reduces the prevalence of liver fibrosis as it reduces HIV viral load because HIV itself can cause liver fibrosis (35). In Nigeria, more than 50% of patients with HIV infection do not experience viral suppression (36). Viral load was not determined for the patients in this study. Hence one may surmise that the patients may not have experience viral suppression and the HIV can cause liver fibrosis leading to the observed higher prevalence of liver fibrosis. This may explain the finding in this study. MPV values also correlated with some markers of fibrosis (FIB-4 and FCI) in this study. In addition to their primary haemostatic functions, platelets are involved in the pathogenesis of infectious diseases (37). Platelets are depleted during an inflammatory or infectious phase as they bind to and occasionally internalize microbes (38). By binding infectious substances and presenting them to neutrophils and/or cells of the reticulo-endothelial system, platelets serve as circulating sentinels that enable their use (39-41). Following the fast release of immature platelets from the spleen or bone marrow, this may cause changes in platelet diameter in response to the infection, which would raise mean platelet volume (MPV) (42). MPV increases as an inflammatory marker during the early stages of infections (37).

The finding that patients with HIV infection irrespective of treatment status have liver fibrosis agrees with previous reports (30, 43).

Conclusion

Liver fibrosis is present in asymptomatic patients with HIV infection, and use of surrogate markers of inflammation and fibrosis can assist in its detection.

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Conflict of Interest

The authors declare no conflict of interest.

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The research received no external funding.

Ethics

This study was approved by Edo State Ministry of Health, Benin City, Edo State, Nigeria, in a letter with ref HM.1208./702

Authors' contributions

All authors were involved in conception and design of the study as well as in data generation. GAA and RO were involved in data analysis. GAA, NLI and RO drafted the manuscript. All authors approved the final draft.

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