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Concordance of Cancer-Associated Cytokines and Mitochondrial DNA Deletion Mutations between Individuals with Hepatocellular Carcinoma and People Living with HIV

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Simple Summary: Hepatocellular carcinoma (HCC) is a major contributor to global cancer-related deaths, including among people living with HIV (PLWH), despite not being an AIDS-defining cancer. The increasing HCC incidence in PLWH is closely linked to chronic HIV infection, which triggers the expression of fibrogenic, cirrhotic, and tumorigenic factors. However, our understanding of how these factors contribute to HCC risk in PLWH is limited. Results revealed similar secretion patterns of specific inflammatory cytokines in PLWH compared to HCC participants without HIV. Additionally, both groups exhibited high levels of mtDNA deletions. These results shed light on the underlying risks associated with HCC development in PLWH and suggest the potential use of these cytokines as biomarkers for HCC surveillance among PLWH.

Abstract: Hepatocellular carcinoma (HCC) is a non-AIDS-defining cancer closely tied to the chronic HIV infection and associated with the release of inflammatory cytokines, immune system dysfunction, and genetic alterations within mitochondria. However, our understanding of how these factors contribute to HCC risk in PLWH is limited. The objective of the study was to ascertain the differential secretion of cytokines and mitochondria DNA (mtDNA) deletion in PLWH, and individuals diagnosed with HCC without HIV. A cross-sectional study was conducted with PLWH and HCC participants from the Korle-Bu Teaching Hospital. Plasma and peripheral blood mononuclear cells (PBMCs) were isolated from whole blood. The plasma samples were used to measure cytokines using ELISA and Luminex techniques. We determined mtDNA deletions from PBMCs. We found that the secretion of the cytokines TGF-β, FGF-2, IL-8, TNF-α, VEGF, and RANTES implicated in the pre-cancer, initiation, and early stages of HCC were similar in PLWH compared to HCC participants without HIV. PBMCs of PLWH exhibited high mtDNA deletion (60%) comparable to HCC participants without HIV (64%). These findings underscore the underlying risks associated with HCC development in PLWH. There is a need for HCC surveillance among PLWH and these cytokines could be used as biomarkers.

Keywords: Hepatocellular carcinoma (HCC); Human immunodeficiency virus (HIV); hepatitis; inflammatory cytokines; mitochondria DNA deletion

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1. Introduction

People living with HIV (PLWH) are at high risk of developing acquired immunodeficiency syndrome (AIDS)-defining cancers such as Kaposi's sarcoma, non-Hodgkin lymphoma, and cervical cancer. Antiretroviral therapy (ART) has reduced the incidence of AIDS-defining cancers and reduced morbidity and mortality, leading to longer life expectancy [1,2]. Despite the benefits of ART, the incidence of non-AIDS-defining cancers in PLWH such as hepatocellular carcinoma (HCC), Hodgkin's lymphoma, skin melanoma, oral, lung, and anal cancers is increasing [1]. The mortality from non-AIDS-defining cancers between 2001 and 2015 was estimated at 9.2% compared to 5% of AIDS-defining cancers in a study conducted in the United States of America [3]. Furthermore, published data from a multicenter study in Europe on PLWH showed 15% mortality associated with non-AIDS-defining cancers [4].

HCC or liver cancer is the sixth most common cancer and the third leading cause of cancer deaths globally. In 2020, over 900,000 new cases were reported worldwide, with the highest burden in Asia and Africa [5,6]. HCC is on the rise among PLWH with an estimated 72% increase in incidence rate and a higher risk among PLWH aged 50 years or older [7]. A recent retrospective study in the USA and Canada estimated the prevalence of HCC between 0.1 to 0.5% in PLWH [7,8]. However, other studies conducted within HIV endemic regions in South Africa, Uganda, and Kenya estimated a prevalence of 18 to 22% showing the enormous burden of HCC cases in PLWH in Sub-Saharan Africa [9–11]. PLWH with chronic hepatitis B or C virus (HBV or HCV respectively) coinfection are at a higher risk of developing HCC [12]. HBV and HCV infections are more predominant in PLWH [13]. Moreover, PLWH with HBV and/or HCV coinfections have a rapid progression to end-stage liver disease compared to individuals with HBV and HCV monoinfections [8,14]. These viruses establish chronic infections and the host immune response to curtail the infection leads to the persistent production of cytokines [15].

Cytokines, which are modulated by the host immune system are secreted to regulate cellular stresses such as infection, inflammation, and carcinogen-induced injury [16,17]. Cytokines are classified as pro- and anti-inflammatory. The imbalance between pro- and anti-inflammatory cytokines in chronic infections results in chronic inflammation. The state of chronic inflammation is critical in the formation, development, progression, and metastasis of cancers [16,18,19]. Although cytokines are linked to the development of several cancers, certain cytokines are markers for specific cancers. For instance, IL-6, TGF-β, FGF-2, MCP-1, and VEGF have been linked to the pre-cancer or initiation (formation) of HCC. The developmental or early stage HCC has been associated with VEGF, GDF-15, RANTES, and OPN, while interleukin-10 (IL-10), MIP-3, and IL-37b are makers for the advanced stage of HCC [16,20]. Other studies have reported tumor necrosis factor-alpha (TNF-α) as a key marker in cirrhosis and HCC development correlating with the secretion of IL-6 [20]. In addition, the secretion of TNF-α is implicated in the production of reactive oxygen species (ROS) by the mitochondria which results in oxidative stress and nucleotide damage leading to mitochondrial DNA (mtDNA) mutagenesis [21]. Interestingly, there are several inflammatory cytokines produced in PLWH resulting in chronic immune activation and inflammation. Furthermore, there is preponderance of data to suggest that HIV increases the risk of cancer, though the underlying mechanisms are not well-understood. Could pro-inflammatory cytokines play a role in the increased incidence of non-AIDS-defining cancers in PLWH?

The purpose of this study was to investigate the differential secretion of cytokines and mitochondria deletion in PLWH and individuals with HCC without HIV infection. We hypothesize that PLWH secrete cytokines that result in chronic systemic inflammation and immune dysregulation responsible for the increased risk of HCC. Delineating the cytokines differentially secreted between PLWH and individuals with HCC could be crucial in screening HCC in PLWH and facilitating appropriate care and treatment.

2. Methods

2.1. Study design and study population

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This was a cross-sectional study of individuals diagnosed with HCC (HCC+) without HIV infection and PLWH (HIV+) receiving care at the Section of Gastroenterology and the Fever's Unit, respectively at the Korle-Bu Teaching Hospital (KBTH), Accra, Ghana. Study participants were recruited on convenience during their routine clinic visits between June and September 2022. HCC participants with fatty or alcohol-associated liver disease were excluded. The study protocol was reviewed and approved by the Institutional Review Boards of the KBTH and Noguchi Memorial Institute for Medical Research (NMIMR).

2.2. Data collection and sample collection

Participants 18 years and above were consented and recruited. Each participant gave a written informed consent. At enrollment, 50 ml of blood was collected from each participant. Participant's medical records and treatment including history of infection, years on ART treatment, HIV viral load, HIV CD4 count, and liver function tests were reviewed and recorded. A study questionnaire administered by the study research assistants was used to obtain demographic and socio-economic data including age, sex, educational background, history of cancer in the family, and history on smoking and alcohol intake. Data was entered and managed using the Research Electronic Data Capture (REDCap) software [22].

2.3. Blood separation

Plasma and peripheral mononuclear blood cells (PBMCs) were separated from whole blood through density gradient centrifugation using Ficoll® as previously described [23]. The plasma was stored at a -20°C while the PBMCs were cryopreserved in freezing medium and stored in liquid nitrogen.

2.4. Serological testing of HBV and HCV

All Participants were screened for HBV (Acro Biotech, California, USA) and HCV (Acro Biotech, California, USA).

2.5. Cytokine profile

Plasma from each participant was assayed for cytokines using ELISA and Luminex assays according to manufacturer's instructions [24,25]. Macrophage inflammatory protein 3 (MIP-3), osteopontin (OPN), growth differentiation factor 15 (GDF-15), and transforming growth factor beta (TGF-β) were measured using ELISA (Sigma, Darmstadt, Germany). Luminex assay (MILLIPLEX MAP, Millipore, Darmstadt, Germany) was used to measure the expression levels of the following cytokines—Fibroblast growth factor 2 (FGF-2), Interferon gamma (IFN- γ), interleukin (IL)-2, IL-6, IL-8, IL-10, IL-12p70, IL-12p40, IL-18, interferon-gamma induced protein 10 (IP-10/CXCL10), monocyte chemoattractant protein 1 (MCP-1), tumor necrosis factor (TNF) - alpha (TNF-α), vascular endothelial growth factor (VEGF), and regulated upon activation, normal T cell expressed and secreted (RANTES/CCL5). The runs were performed in duplicates.

2.6. Mitochondrial mutations

To assess the mtDNA common deletion (4977 deletion), nested PCR was performed using primers and protocols as previously described [26]. In brief, genomic DNA was extracted from PBMCs using the DNeasy blood and tissue kit (QIAgen, Hilden, Germany). Two pairs of primers were used for the detection of the 4,977-bp deletion: The round 1 primers were 1F: AACCACAGTTTCATGCCCATC; and 1R: TGTTAGTAAGGGTGGGGAAGC; and the round 2 primers were 2F: ACCCTATTGCACCCCCTCTAC; and 2R: CTTGTCAGGGAGGTAGCGATG. The PCR condition set used for the nested PCR was pre-denaturation at 94°C for 5 min; then 30 cycles at 94°C for 10 s, 58°C for 45 s and 72°C for 50 s; and a final extension at 72°C for 10 min. The PCR products were then electrophoresed on 2% agarose gel and the 4,977-bp deletion was indicated by the presence of a 358-bp band.

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2.7. Data analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS; IBM Corporation, Armonk, NY) and GraphPad Prism 9 (Dotmatics, Boston, USA). Categorical variables were presented as frequency and percentage, while continuous variables were expressed as median and interquartile range (IQR) with confidence interval (CI). Chi-square and/or regression analysis were used in determining risk-associated factors. Statistical significance was considered $p < 0.05$.

3. Results

3.1. Demographic and disease characteristics of study participants

We enrolled a total of 93 participants — 33 had HCC without HIV (HCC+) and 60 were PLWH without HCC (HIV+) as shown in Table 1. Females made up 63%, and the mean age of study participants was 50 ± 13 years. About 54% were aged 50 years and above. The highest level of education was junior high school for two-thirds of participants (65%). Nearly half of the participants had a history of alcohol usage. The prevalence of hypertension was higher in HIV+ participants compared to HCC+ participants. All the HCC+ participants were hepatitis B or C positive, whereas 16 out of the 60 (26.7%) PLWH were co-infected with either hepatitis B or C virus. Study participants with HIV only were 44 (73.3%) of the 60 PLWH.

Table 1. Demographic and exposure characteristics of study participants.

	HIV+ N (%) 60 (64.5)	**HCC+ N (%) 33 (35.5)**	**Total N (%) 93 (100.0%)**	**P value**
Gender				
Female	43 (71.7)	15 (45.5)	58 (62.8)	**< 0.01**
Male	17 (28.3)	18 (54.5)	35 (27.2)	
Age				
< 50	27 (45.0)	16 (48.5)	43 (46.2)	**0.31**
≥ 50	33 (55.0)	17 (51.5)	50 (53.8)	
Mean ± SD	51 ± 11	50 ± 15	50 ± 13	
Educational Level				
≤ Junior High School	45 (75.0)	15 (45.5)	60 (64.5)	**< 0.01**
> Junior High School	15 (25.0)	18 (54.5)	33 (35.5)	
History/Exposure				
Family history of cancer	2 (3.3)	5 (15.2)	7 (13.2)	**0.04**
Hypertension	33 (55.0)	13 (39.4)	46 (49.5)	**0.15**
Smoking	1 (1.7)	2 (6.1)	3 (3.2)	**0.26**
Alcohol	30 (50.0)	19 (57.6)	49 (52.7)	**0.48**
Virus Infection				
HCV	6 (10.0)	7 (21.2)	13 (14.0)	**0.14**
HBV	10 (16.7)	31 (93.9)	41 (44.1)	**< 0.01**
Hepatitis B and C coinfection	0 (0.0)	5 (15.2)	5 (5.4)	**< 0.01**

As shown in Table 2, 31 (54%) of the HIV+ participants had been diagnosed of HIV for at least 10 years while 28 (50%) had been on ART for 10 years. Of the HIV+ participants, most of them were categorized as HIV stage 1 (43.6%), had viral loads below 50 copies/ml (78.2%), and had CD4 cell counts above 200 cells (89.4%).

Table 2. HIV-associated characteristic in the HIV+ participants.

Variable	**HIV+**

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	N (%)	*P-value*
Years Diagnosed		
< 10	26 (43.3)	**0.35**
≥ 10	31 (51.7)	
Missing	3 (5.0)	
Years on ART		
< 10	28 (46.7)	**1.00**
≥ 10	28 (46.7)	
Missing	4 (6.7)	
HIV Stage		
1	24 (40.0)	**< 0.01**
2	10 (16.7)	
3	13 (21.7)	
4	8 (13.3)	
Missing	5 (8.3)	
Viral Load		
≤ 50 Median (Range)	43 (71.7) < 20 (< 20 - 30)	**< 0.01**
> 50 Median (Range)	12 (20.0) 5731.5 (52 - 2.5e5)	
Missing	5 (8.3)	
CD4 count		
< 200 Median (Range)	5 (8.3) 159 (21 - 183)	**< 0.01**
200 – 499 Median (Range)	17 (28.3) 383 (202 - 495)	
> 500 Median (Range)	25 (41.7) 721 (502 - 1353)	
Missing	5 (100.0)	

3.2. Liver function of study participants

Liver function tests including alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin, and bilirubin were extracted from participant's medical records. The records showed that the HCC+ participants had higher levels of liver enzymes ALT, AST, and ALP as well as bilirubin compared to the HIV+ participants. However, the albumin level was lower in the HCC+ participants compared to the HIV+ participants (Table 3).

Table 3. Liver function tests (liver enzymes and proteins) in the HIV+ and HCC+ cohorts.

Variable	Study Cohort		P value
	HIV+	HCC+	
	ALT (IU/L)		
Median (Range)	22 (7 - 123)	62 (17 - 553)	**< 0.01**
Normal Range	7 to 55		
	AST (IU/L)		
Median (Range)	32 (17 - 106)	91 (19 - 870)	**< 0.01**
Normal Range	8 to 48		
	ALP (IU/L)		
Median (Range)	99.5 (0 - 323)	207 (91 - 1581)	**< 0.01**
Normal Range	44 to 147		
	Albumin (g/L)		
Median (Range)	41 (4.3 - 267)	35 (21 - 46)	**0.04**
Normal Range	34 to 55		

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Bilirubin (μmol/L)			
Median (Range)	7.9 (0 - 73)	23.1 (4 - 680)	**< 0.01**
Normal Range	1.7 to 20.5		

3.3. Cytokine profile of study participants

We determined the cytokine profile of HCC+ participants compared to HIV+ participants. We screened for 18 cytokines that were either pro-inflammatory or anti-inflammatory. Next, we grouped them into cytokines associated with pre-cancer or the initiation stage of HCC (IL-6, TGF-β, MCP-1, FGF-2, IL-2, IL-8, IL-12p40, IL-12p70, IL-18, IP-10, TNF-α, and IFN-γ), early stage of HCC (OPN, GDF-15, RANTES, and VEGF), and advanced stage of HCC (IL-10, and MIP-3) . As shown in Figure 1, the cytokines associated with the pre-cancer or the initiation stage of HCC, TGF-β, FGF-2, IL-8, and TNF-α were expressed at similar levels in the HIV+ participants compared to the HCC+ participants. However, IL-6, MCP-1, and IP-10 had significantly higher expression in HCC+ participants than in HIV+ participants. Comparison of cytokines implicated in the early stage of HCC showed that OPN was significantly expressed in the HCC+ participants but VEGF, GDF-15 and RANTES were secreted to similar levels compared to that of the HIV+ participants (Figure 2A). More importantly, among the PLWH, the pre-cancer, initiation, and early-stage cytokines were expressed at similar levels between hepatitis positive and negative patients (Figures 1 and 2A). The advanced stage cytokines IL-10 and MIP-3 were significantly expressed in HCC+ participants than the HIV+ participants (Figure 2B).

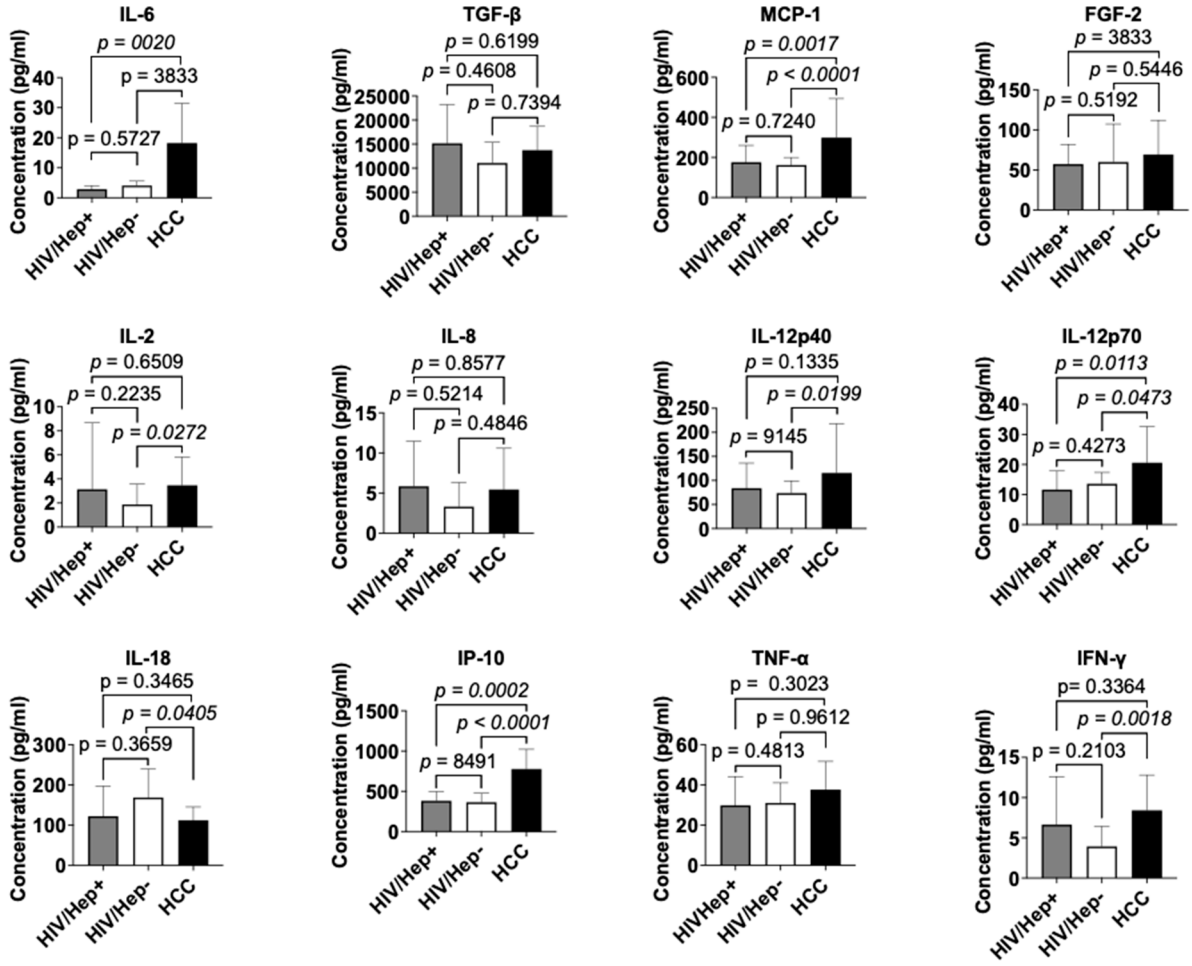

Figure 1. Expression of inflammatory cytokines associated with pre-cancer and initiation of HCC in HCC+ compared to HIV+ participants coinfected with or without hepatitis B and C viruses. The grey bar denotes HIV/Hep+ participants, white bar denotes HIV/Hep- participants, and black bar denotes the HCC+ participants. The sample were run in duplicates, the bar graphs with error bars

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were represented as median and 95% CI respectively. Differences between groups were tested by Mann-Whitney U test and significance was considered at p (p-value) < 0.05.

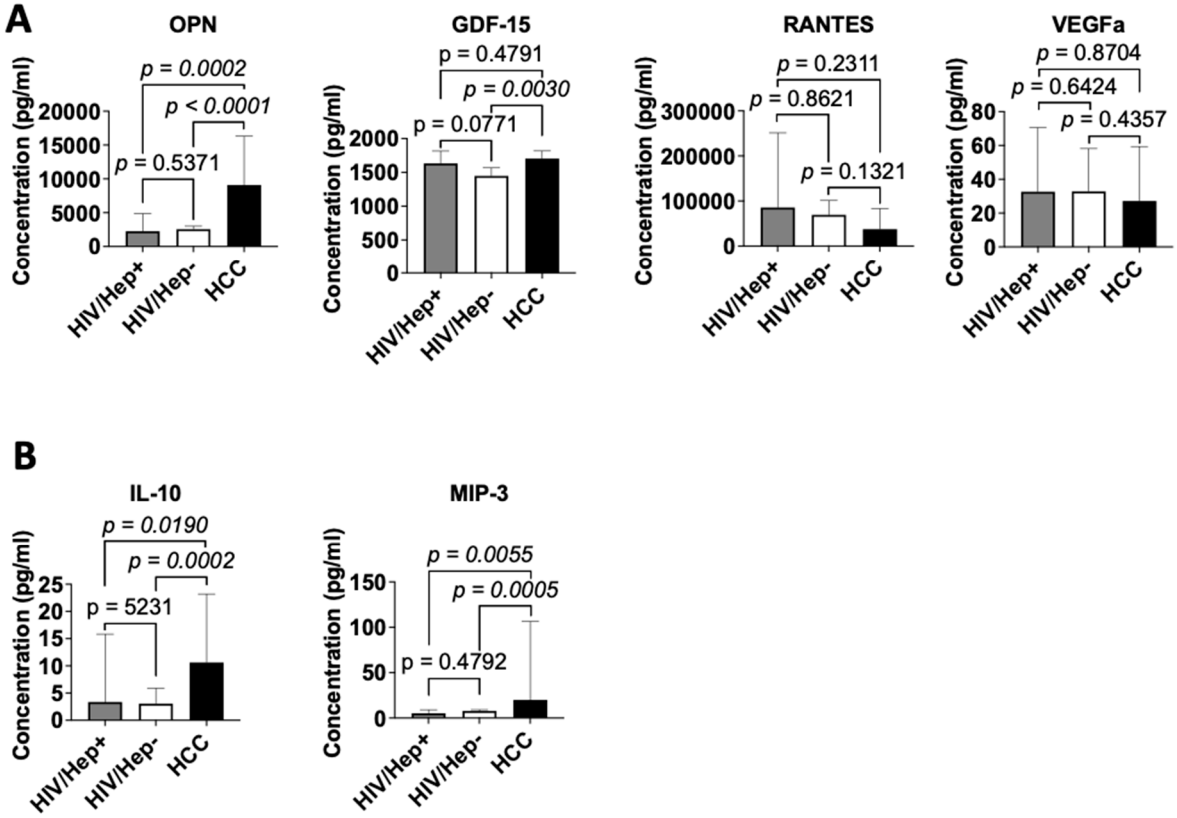

Figure 2. Expression of cytokines implicated in the early and advanced stages of HCC in HCC+ compared to HIV+ participants coinfected with or without hepatitis B and C viruses. (A) Cytokines implicated in the early or developmental stage of HCC. (B) Cytokines implicated in the advanced stage of HCC. The grey bar denotes HIV/Hep+ participants, white bar denotes HIV/Hep- participants, and black bar denotes the HCC+ participants. The sample were run in duplicates, the bar graphs with error bars were represented as median and 95% CI respectively. Differences between groups were tested by Mann-Whitney U test and significance was considered at p (p-value) < 0.05.

3.4. Correlation of cytokine levels in HIV+ participants

We next investigated the correlation among the 19 cytokines screened. As presented in Table 4, the notable pairwise correlations with the HIV+ cohort were 0.89 for IL-12p70 and IL-12p40, 0.87 for RANTES and TGF-β, and 0.85 for TNF-α and TGF-β. There was high correlation of the cytokines implicated in the pre-cancer, the initiation stage of HCC, and the early stage of HCC. Notably, the cytokines including IL-6, TGF-β, FGF-2, VEGF, IL-8, TNF-α, and RANTES exhibited positive correlations with each other. Interestingly, GDF-15 negatively correlated with IL-8, TGF-β, VEGF, OPN, and RANTES in the HIV+ participants.

Table 4. Correlation of cytokines in PLWH. Values in red denote significant correlations of the level of cytokines among individuals living with HIV.

		HIV+																
		IL-6	TGFb	MCP 1	FGF-2	VEGF	OPN	GDF-15	RANTES	IL-10	MIP-3	IP-10	IL-2	IL-8	IL-12p70	IL-12p40	IL-18	TNF-α	IFN-γ
HIV+	IL-6	1																	
	TGF-β	0.52	1																

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MCP1	0.27	-0.06	1															
FGF-2	0.56	0.69	0.05	1														
VEGF	0.37	0.60	0.06	0.46	1													
OPN	0.41	0.29	-0.02	0.11	0.23	1												
GDF-15	-0.13	-0.33	0.17	-0.18	-0.29	-0.26	1											
RANTES	0.37	0.87	0.00	0.55	0.66	0.30	-0.35	1										
IL-10	0.22	0.01	0.17	-0.03	0.02	0.42	0.16	-0.03	1									
MIP-3	0.22	0.09	0.00	0.02	0.11	0.09	-0.12	0.02	-0.07	1								
IP-10	0.08	-0.03	0.38	0.01	0.00	0.03	0.13	-0.03	0.31	0.02	1							
IL-2	0.57	0.20	0.43	0.42	0.29	0.21	0.00	0.22	0.24	-0.06	0.21	1						
IL-8	0.43	0.64	-0.28	0.63	0.32	0.20	-0.28	0.49	-0.14	0.09	-0.07	-0.12	1					
IL-12p70	0.56	0.24	0.52	0.25	0.33	0.29	0.02	0.26	0.47	-0.10	0.32	0.76	-0.10	1				
IL-12p40	0.59	0.26	0.52	0.22	0.25	0.34	-0.04	0.23	0.40	-0.08	0.29	0.72	-0.12	0.89	1			
IL-18	0.24	0.22	0.05	0.11	0.07	-0.12	0.06	0.17	-0.01	-0.11	0.13	0.03	0.35	0.11	0.02	1		
TNF-α	0.52	0.85	-0.08	0.65	0.56	0.23	-0.19	0.68	-0.01	0.06	0.08	0.08	0.68	0.12	0.16	0.27	1	
IFN-γ	0.38	0.06	0.04	0.46	0.22	0.05	0.06	0.07	0.06	-0.04	0.10	0.73	0.00	0.38	0.26	0.12	0.00	1

3.5. Mitochondrial mutations among the cohorts

Mitochondrial mutagenesis has been associated with the development of cancers [27]. Mitochondrial deletion mutations are more prevalent than point mutations [28]. We therefore investigated mitochondrial deletion mutations in the PBMCs of HCC+ and HIV+ participants. We observed a high rate of mtDNA deletion in both cohorts; 60% and 64% in HIV+ and HCC+ participants, respectively. Overall, we observed that mtDNA deletion was associated with increase in age, and male gender. There was no association between mtDNA deletion and history of alcohol usage (Table 5). Risks analysis showed that age above 50 (OR 3.3; CI 1.1 - 9.8), males (OR 4.5; CI 1.1 - 17.8), diagnosed with HIV over 10 years (OR 3.3; CI 1.1 - 10.0), use of ART over 10 years (OR 2.1; CI 0.7 - 6.2), and viral loads more than 50 copies (OR 2.2; CI 0.5 - 9.1) were associated with mtDNA deletions in the HIV+ cohort. HIV-hepatitis coinfected participants (69%) had a 1.7 times likelihood for mtDNA mutagenesis compared to their HIV-monoinfected participants (57%).

Table 5. Frequency of mtDNA deletion among the HIV+ and HCC+ cohort.

Variable	Study Cohort			
	HIV+	**HCC+**	**Total**	**P value**
mtDNA mutation				
Present	36 (60.0)	21 (63.6)	57 (61.3)	**0.730**
Absent	24 (40.0)	12 (36.4)	36 (38.7)	
P value	**0.03**	**0.03**	**< 0.01**	
		Age		
18-39	3/7 (42.9)	4/7 (57.1)	7/14 (50.0)	**0.59**
40-49	9/20 (45.0)	5/9 (55.6)	14/29 (48.3)	**0.60**
≥ 50	24/33 (72.7)	12/17 (70.5)	36/50 (72.0)	**0.87**
P value	**0.13**	**0.42**	**0.07**	
		Sex		
Female	22/43 (51.2)	9/15 (60.0)	31/58 (53.4)	**0.55**
Male	14/17 (82.4)	12/18 (66.7)	26/35 (74.3)	**0.29**
P value	**0.03**	**0.69**	**0.05**	
		Alcohol history		
Yes	18/30 (60.0)	10/19 (52.6)	28/49 (57.1)	**0.61**
No	18/30 (60.0)	11/14 (78.5)	29/44 (65.9)	**0.23**

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P value	1.00	0.13	0.39	

Table 6. Risks associated with mtDNA deletions in PLWH.

Variable	mtDNA deletion		OR (CI)	P value
	Yes (%)	No (%)		
Age				
< 50	12 (44.4)	15 (55.6)	1	
≥ 50	24 (72.7)	9 (27.3)	3.3 (1.1 – 9.8)	**0.03**
Sex				
Female	22 (51.2)	21 (48.8)	1	
Male	14 (82.4)	3 (17.6)	4.5 (1.1 – 17.8)	**0.03**
Years Diagnosed				
< 10	11 (42.3)	15 (57.7)	1	
≥ 10	22 (71.0)	9 (29.0)	3.3 (1.1 – 10.0)	**0.03**
Years on ART				
< 10	14 (50.0)	14 (50.0)	1	
≥ 10	19 (67.9)	9 (32.1)	2.1 (0.7 – 6.2)	**0.18**
Viral Load				
≤ 50	25 (58.1)	18 (41.9)	1	
> 50	9 (75.0)	3 (25.0)	2.2 (0.5 – 9.1)	**0.29**
CD4 count				
< 200	1 (20.0)	4 (80.0)	0.1 (0.0 – 1.5)	**0.10**
200 – 499	13 (76.5)	4 (23.5)	1.8 (0.5 – 7.3)	**0.39**
> 500	16 (64.0)	9 (36.0)	1	
Hepatitis status				
Positive	11 (68.8)	5 (21.2)	1.7 (0.5 -5.6)	**0.47**
Negative	25 (56.8)	19 (43.2)	1	

4. Discussion

The chronic inflammation state of HBV or HCV stimulates the continuous secretion of cytokines that contributes to HCC development. Moreover, HIV infection leads to chronic inflammation with the secretion of several cytokines (proinflammatory or anti-inflammatory) that could initiate the development of cancers [29]. Consequently, with the increased incidence of non-AIDS-defining including hepatocellular carcinoma, we raised the question: Could pro-inflammatory cytokines play a role in the increased incidence of non-AIDS-defining cancers such as HCC in PLWH? We found high levels of inflammatory cytokines TGF-β, FGF-2, IL-8, TNF-α, VEGF, and RANTES, and mutagenesis of mtDNA in PLWH. These are cytokines implicated in the pre-cancerous, initiation and early stages of HCC. Importantly, the study offered preliminary evidence indicating a resemblance between the secretion patterns of these inflammatory cytokines in PLWH and those observed in HCC patients, thereby underscoring the potential risks of HCC development among PLWH.

Comparison of the levels of cytokines implicated in liver fibrosis, cirrhosis, or the initiation of HCC in HIV+ participants were similar to those in HCC+ participants with the exception of IL-6 and MCP-1. The cytokines TGF-β, FGF-2, IL-8, and TNF-α as observed in the HCC patients and PLWH were consistent with studies implicating these cytokines in hepatic injury and the initiation of HCC in PLWH [30–33]. Moreover, studies have further shown that HIV-infected PBMCs stimulates the expression of pro-inflammatory, pro-fibrogenic, angiogenic, and proliferative cytokines including IL-6, VEGF, and TGF-β in hepatic stellate cells [34]. The secretion of these cytokines exhibited a positive correlation among the HIV participants, which aligns with findings from other studies indicating a correlation between the cytokines TNF-α, IL-6, and IL-8 in PLWH coinfected with hepatitis, as well as in individuals with chronic hepatitis. [32,35]. TGF-β is anti-inflammatory and involved in inhibiting apoptosis, while TNF-α, IL-8, FGF-2, and VEGF are involved in hepatic injury or cirrhosis through

cell proliferative and angiogenic activities [16,36]. High levels of IL-6 and MCP-1 correlate with worse prognosis for cirrhotic and HCC patients hence suggested as biomarkers to track the development of HCC [37–39]. This implies that the low levels of IL-6 and MCP-1 in the HIV+ participants compared to the HCC individuals, could be monitored for liver prognosis. Additional studies are necessary to determine if IL-6 together with cytokines such as MCP-1 could be used to monitor the initiation and progression of HCC in PLWH.

The observed expression of VEGF and RANTES, both linked to the early stages of HCC development, is consistent with previous findings [40,41]. Studies have suggested that VEGF and RANTES could predict the presence of HCC and high levels of these cytokines could be important as prognostic factor in determining the survival of HCC patients [42,43]. Notably, VEGF is closely tied to angiogenesis, while RANTES has been implicated in facilitating metastasis [42,43]. These preliminary findings underscore the potential role of VEGF and RANTES as critical players in the early progression of HCC, thereby warranting further investigation into their mechanistic contributions and potential clinical implications.

Furthermore, consistent with other studies, our findings showed that MIP-3 and IL-10 secretions are associated with the advanced stage of HCC [44]. The study findings showed low secretion of MIP-3 and IL-10 among PLWH compared to HCC patients. This finding is consistent with other studies showing high levels of MIP-3 and IL-10 in HCC patients compared to their cirrhotic or non-HCC counterparts [44–46]. The secretion and expression of MIP-3 is further associated with the developmental stages or grades of the liver tumors [47]. This suggests that the study's HIV+ participants were likely not having any advanced HCC. MIP-3 and IL-10 are associated with chronic infections with anti-inflammatory effects in immune regulatory, angiogenesis and tumorigenesis [46,48].

Consistent with other studies, our findings revealed a high rate of mtDNA mutations (deletions) in PBMCs from both HIV+ and HCC+ participants [49–51]. In addition, the study results suggest that hepatitis infection contributes to high mtDNA deletions in HIV-hepatitis coinfected participants compared to HIV-monoinfected participants as demonstrated in another study [51]. Mitochondrium is the chief powerhouse of the cell with its function designed to meet the energy and metabolic needs of the cell [27]. However, several environmental conditions including chronic infections as established in our study participants by the HIV and hepatitis viruses, could disrupt the functions of the mitochondria [51,52]. The most common causes of disruption of mitochondrial functions are mtDNA mutations, deletions, and impaired DNA replication. The accumulation of mtDNA mutagenesis is a hallmark of mitochondrial dysfunction which is implicated in carcinogenesis including HCC [52]. Subsequently, mitochondrial dysfunction results in the generation of mitochondrial reactive oxygen species (ROS). The mitochondrial ROS can result in further damage to the structure and functions of the mitochondria, other organelles, cells, tissues, and organs contributing to carcinogenesis. Furthermore, chronic infections with the HIV and hepatitis B and C viruses as observed in the HIV and HCC participants stimulate the secretion of cytokines such as TNF-α. The secretion of TNF-α stimulates the production of ROS resulting in oxidative stress. These ROS-generated oxidative stress results in nucleotide damage leading to mtDNA deletions and subsequently affecting the genome integrity, apoptosis, and promoting the outgrowth of cancerous cells [53,54]. The biology and the interactions of HIV and hepatitis B and C viruses on mtDNA mutations and their implications in the development of HCC is not well understood. Therefore, further studies would be important to determine the role of chronic hepatitis infection in the initiation and progression of HCC in HIV-hepatitis coinfected patients compared the HIV-monoinfected patients.

The study sought to determine factors contributing to the increased incidence of HCC among PLWH in the Sub-Saharan African region. Despite the study determining the differential secretion of cytokines between PLWH and HCC individuals, as well as mitochondria mutagenesis, it is important to acknowledge the inherent limitations within this study. Firstly, the research was conducted as an exploratory study, resulting in a relatively small sample size. The cross-sectional study design, though informative, would benefit from the establishment of a larger cohort for longitudinal observation to thoroughly examine the dynamic relationship between inflammatory cytokines and HCC development in PLWH. Secondly, inclusion of a well-matched healthy control group, factoring

in gender and age, would establish baseline levels of cytokine secretion within PLWH. Thirdly, the absence of ultrasound screening to assess liver conditions in HIV-positive participants, including potential factors like fibrosis and cirrhosis, is a noteworthy limitation. Correlating such factors with cytokine secretion patterns could provide valuable insights. Finally, the study did not assess the correlation between cytokine levels and mtDNA mutagenesis with the viral load of HBV or HCV.

5. Conclusion

In essence, HIV infection, regardless of hepatitis coinfection, appears to trigger the release of cytokines associated with fibrogenesis, cirrhosis, liver tissue tumorigenesis, and mutagenesis of mtDNA, potentially initiating HCC among PLWH. The study presents initial evidence that the profile of these inflammatory cytokines released in PLWH resembles that found in HCC patients, implying an elevated risk of HCC development in PLWH. These findings shed light on the underlying risks of HCC in PLWH and underscore the importance of HCC surveillance within this population. Additional research is imperative to comprehensively investigate and elucidate the roles of cytokines and mtDNA mutations in HCC development, as well as their implications for other cancers within the context of HIV infection and concurrent coinfections with opportunistic viruses.

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Data Availability Statement: The data presented in this study are available upon request from the corresponding author. The data are not publicly available due to ethical restrictions and information that could compromise the privacy of the study participants.

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