Assessment of the Level of Cytokines in People living with HIV, HCV and HIV/HCV Individuals in Port Harcourt Metropolis, Rivers State, Nigeria

Baeka G. B.[1*] and Oloke J. K[2]

1. Department of Microbiology, Rivers State University, P.M.B 5080, Nkpolu-Oroworukwo, Port Harcourt, Nigeria.
2. Department of Department of Pure and Applied Biology (Microbiology Unit), Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

* Corresponding author: baeka.barinuaka@ust.edu.ng

Abstract: Cytokines, play a crucial role regulating the immune response and coordinating the communication between cells of the immune system and other tissues. The co-infection of HIV/HCV in patients compared to mono infections has been discovered to have reduced anti-inflammatory cytokines in peripheral blood mononuclear cells (PBMC) and dehydroepiandrosteronesulphate in plasma. The aim of this study therefore, is to compare the level of some inflammatory cytokines such as the Interleukine 1B, 6,12, Tumour Necrosis Factor α in HIV-positive, HCV (mono infection) and HIV/HCV co-infected individuals. Blood samples were collected from 1250 HIV-negative individuals and 550 HIV-positive patients attending the antiretroviral clinic in Port Harcourt, Rivers State, Nigeria, between November 2018 and October 2019. Enzyme linked Immunosorbent Assay (ELISA) was used to screen for HCV antibodies (for detection of HCV mono-infection and HIV/HCV co-infection) and determination of cytokines levels. Thirty-six HIV-negative infected individuals (2.88%) tested positive for HCV antibody and, twenty-four (4.4%) tested positive for HCV antibody in the HIV-positive individuals. The IL1β in HIV mono-infection was significantly ($P < 0.05$) lower. The IL 6 was significantly higher ($P < 0.05$) among HIV mono infection. The HIV/HCV showed a higher significant level ($P < 0.05$) for IL12, while there was no significant difference ($P > 0.05$) across the groups for TNFα. Findings from this study discovered a higher level of cytokines across the three groups than in previously reported findings. It is recommended that measures be put in place to control pollutions which may lead to cytokine increase.

Key word: Co-infection, Cytokines, ELISA, Interleukines

INTRODUCTION

Cytokines, a group of immunological markers are a diverse group of small proteins that are secreted by immune cells and other cells in response to an immune stimulus or other biological signals (Friedman *et al*., 2006). They play a crucial role in regulating the immune response and coordinating the communication between cells of the immune system and other tissues in the body (Schwartz *et al.,* 2020). Hepatitis C virus has been discovered to inhibit the production of interferons (IFNs), which are key antiviral cytokines and often leads to chronic inflammation in the liver (Bader *et al.,* 2024). This inflammatory response involves various cytokines, including interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha) which contributes to liver damage and fibrosis over time (Heidrich *et al*., 2019). The release of pro-inflammatory cytokines can lead to liver cell injury and fibrosis, which, if left unchecked, can progress to cirrhosis (Hora and Wuestefeld, 2023). Human Immunodeficiency Virus infection on the other hand has been found to be associated with dysregulated cytokine production and signaling, leading to elevated levels of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, and reduced levels of anti-inflammatory cytokines (Xu *et al.,* 2019). Cytokines are essential mediators of the immune response, and their dysregulation has been implicated in the pathogenesis of HIV (Koyanagi *et al*., 2022). The co-infection of HIV/HCV in patients compared to mono infection of HCV have been discovered to have a greatly reduced anti-inflammatory cytokines such as IFN-y, and IL-4, and so on in peripheral blood mononuclear cells (PBMC) and dehydroepiandrosteronesulphate in plasma (Lee *et al.*, 2006).

The aim of this study is therefore to compare the level of some inflammatory cytokines such as the Interleukin 1B, Interleukin 6, Interleukin 12, the Tumor Necrosis Factor

alpha in people living with HIV, HCV mono infected people and HIV/HCV co-infected individuals.

MATERIALS AND METHODS

Sample collection: A cross-sectional study was carried out at the Braithwaite Memorial Hospital, Port Harcourt, Nigeria, between November 2018 and October 2019. Five milliliter of blood was obtained from 1250 patients from the out-patient ward who were negative to HIV and from 550 people living with HIV attending the antiretroviral clinic, Adults (18–65 years) who provided written informed consent. The protocol used for the study was in line with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. The patients were enrolled into three groups: (1) HIV/HCV co-infected individuals on antiretroviral therapy for at least six months, confirmed HCV antibody–positive and hepatitis B–negative without clinical hepatitis, and (2) HCV mono-infected outpatients who were HIV-negative and hepatitis B–negative. (3) HIV mono infected, negative to both HCV and Hepatitis B. Demographic data (age, sex, occupation) and relevant medical history were collected using a detailed questionnaire. The blood samples collected were analyzed for some inflammatory cytokines such as ILB, IL6, IL12 and Tumour Necrosis factor α level in patients positive to HCV, HIV/HCV Co-infected and also in people living with HIV.

Study area: Port Harcourt is a coastal city in Rivers State, southern Nigeria, where crude oil is mined and refined both legally and illegally. The Braithwaite Memorial Hospital now known as the Rivers State University Teaching Hospital is a referral center for infectious diseases in Port Harcourt.

Sample processing: Each participant provided a single, non-fasting 5 ml venous blood drawn into an ethylenediaminetetraacetic acid (EDTA) tube, samples were centrifuged at 3,000 × g for 10 minutes; plasma was aliquoted and stored at –70 °C. Once the target sample size was reached, all plasma aliquots were transported on ice to the University College Hospital (UCH) laboratory in Ibadan for analysis.

Hepatitis C Virus Antibody and the cytokine detection were carried out using a third-generation ELISA kit (Melsin Medical Co., Limited) following manufacturer's instructions. Absorbance was read at 450 nm, and samples were classified based on the kit's cut-off as prescribed by Cestin *et al.,* (2018).

Ethical approval: The ethical approval on the strength on which this study was carried out, was obtained from the Rivers State ethical health committee (Approval No. RSHMB/RSHREC/11.21/VOL8/127).

Statistical analysis: Analyses were performed in Stata v16 (Stata Corp, College Station, TX, USA). Continuous variables are reported as mean ± standard deviation or median (interquartile range), depending on normality (Shapiro–Wilk test). Cytokine levels across HIV/HCV co-infected, HCV mono-infected, and HIV-positive only groups were compared using the Kruskal–Wallis test ($p < 0.05$ considered significant).

RESULTS

Among 1,250 HIV-negative individuals, 36 (2.9 %) were HCV-antibody positive; among 550 HIV-positive individuals, 24 (4.3 %) were HCV-antibody positive."

The mean value of the IL 1B for the people living with HIV was 6.67mg/dl ±11.55, HCV mono infected patients was 17.629mg/dl ± 17.224. While the mean value for the HCV/HIV co infected patients' was17.754mg/dl ± 4.712. Using the Kruskal-Wallis test for the comparison of level of human Interleukin 1B between the groups of patients, there was a significant difference in the level of IL 1B between people living with HIV and people mono infected with HCVand also between people living with

HIV and those co-infected with HIV/HCV as shown in Table 1.

The mean value of the IL 6 for people living with HIV was 6.67mg/dl ±5.51, the HCV mono infected patients' was 2.092mg/dl ± 3.423. While the mean value for the HIV/HCV co infected patients' was 1.303mg/dl ± 1.803. Comparing the level of human interleukin 6 between the groups of patients infected with HIV, HCV, and HIV/HCV using Kruskal-Wallis test, showed a significant difference between the HIV group and the HCV mono infected group and the HIV group and the HIV/HCV co-infected group as shown in Table 2.

The mean value of the IL 12 for people living with HIV was 6.67mg/dl ±10.69, the HCV mono infected patients was 65.302mg/dl ± 14.323. While the mean value for the HCV/HIV co infected patients was 99.267mg/dl ± 13.118. Comparison of the level of human Interleukin 12 between the groups of patients infected with HIV, HCV, and HIV/HCV using Kruskal-Wallis test as shown in Table 3 shows a significant difference in the IL12 level between the HIV group and the HIV/HCV co-infected group and also between the HCV mono infected group and the HIV/HCV co-infected group.

The mean value of the Tumour Necrosis Factor α for people living with HIV was 6.67mg.d±9.87, the HCV mono infected patients was 166.667mg/dl ± 36.614. While the mean value for the HIV/HCV co infected patients was 166.417mg/dl ± 34.631. Table 4 shows the comparison of the level of the Tumor Necrosis Factor alpha between the different groups of patients'using Kruskal-Wallis test shows that there was no significant difference across the groups with a *P* value > 0.005.

Table 1: Comparison of level of human interleukin 1B between the groups of patients infected with HIV, HCV, and HIV/HCV

Level of human interleukin 1B	(a) HIV Patients (n=20) x̄±SD	(b) HCV Patients (n=36) x̄±SD	(c) HIV/HCV Patients (n=24) x̄±SD	K-value	p-value	Wilcoxon Post Hoc Test
Optical Density (OD)	0.079±0.005	0.100±0.069	0.101±0.018	16.614*	<0.01	a&b, a&c
Conc (pg/ml)	12.560±1.146	17.629±17.224	17.879±4.506	17.626*	<0.01	a&b, a&c

*Post Hoc Test: a&b means Samples a is significantly different from Sample b, a & c means Sample a is significantly different from Sample c,*significant at 1%*

Table 2: Comparison of level of human interleukin 6 between the groups of patients infected with HIV, HCV, and HIV/HCV

Level of human interleukin 6	(a) HIV patients (n=20) x̄±SD	(b) HCV patients (n=36) x̄±SD	(c) HIV/HCV patients (n=24) x̄±SD	K-value	p-value	Wilcoxon Post Hoc
Optical Density (OD)	0.113±0.034	0.099±0.026	0.089±0.015	8.219*	<0.05	a&b, a&c
Conc (pg/ml)	3.932±4.672	2.092±3.423	1.303±1.803	6.846*	<0.05	a&b, a&c

Post Hoc Test: a&b means Samples a is significantly different from Sample b
** Significant at 5%*

Table 3: Comparison of level of human interleukin 12 between the groups of patients infected with HIV, HCV, and HIV/HCV

Level of human interleukin 12	(a) HIV Patients (n=20)	(b) HCV Patients (n=36)	(c) HIV/HCV Patients (n=24)	K-value	p-value	Wilcoxon Post Hoc
	$\bar{x}$±SD	$\bar{x}$±SD	$\bar{x}$±SD			
Optical Density (OD)	0.714±0.0.155	0.781±0.156	1.155±1.244	2.951**	<0.01	a&c, b&c
Conc (pg/ml)	59.175±14.089	65.303±14.324	99.267±113.118	12.951**	<0.01	a&c, b&c

*** significant at 1%*

Post Hoc Test: a&b means Samples a is significantly different from Sample b

Table 4: Comparison of level of the tumor necrosis factor alpha between the groups of patients infected with HIV, HCV, and HIV/HCV

Tumor necrosis factor α	(a) HIV Patients (n=20)	(b) HCV Patients (n=36)	(c) HIV/HCV Patients (n=24)	K-value	p-value	Wilcoxon Post Hoc
	$\bar{x}$±SD	$\bar{x}$±SD	$\bar{x}$±SD			
Optical Density (OD)	0.832±0.080	0.913±1.831	0.912±0.173	3.027	>0.05	
Conc (pg/ml)	150.300±15938	166.667±36.614	166.417±34.631	3.027	>0.05	

Post Hoc Test: a&b means Samples a is significantly different from Sample b

DISCUSSION

In this study, the Interleukin 1B, 6, 12 and the Tumour Necrosis Factor alpha level in HIV, HCV mono infected patients and patients co-infected with HIV/HCV was detected. The mean values and the significant difference for each of the interleukins and the tumour necrosis factor for the HIV, HCV mono infected patients and the HIV/HCV co-infected patients were revealed.

One of the best-known interleukins is IL-1B, which plays a key role in initiating the inflammatory response (Abou-Fayssal *et al.,* 2021). The level of the IL 1B was determined among people living with HIV, Patients' mono infected with HCV and those co-infected with HIV/HCV showed significant increase in HIV/HCV co-infected people and HCV mono infected patients compared with the level found in people living with HIV. This suggests that HCV infection produces a higher level of IL 1B than HIV infection and that the co-infection of both viruses further increases the production of IL 1B.

The standard level of Interleukin 1B in apparently healthy individuals as stated by O'Neil *et al.* (2013) was given to be between 0.5 – 12pg/ml. The level of Interleukin 1B in patients infected with HIV in this study was slightly higher than the normal range prescribed by O'Neil *et al.* (2013), while the levels found in HCV mono infected patients and HIV/HCV co-infected patient were significantly higher than the normal range. From the work done by Libra *et al*. (2005), It was discovered that the level of IL 1B was 7.0pg/ml in HCV mono infected patients and that found in the study carried out by Jing *et*

al. (2020) for HIV/HCV co infection was 10.84pg/ml which were both within the normal standard level of IL 1B. The findings from this study disagrees with the both studies, showing higher values for both HCV and HIV/HCV groups. This could be due to environmental factors such as air pollution caused by crude oil refining, since Port Harcourt is currently heavily polluted by black soot from illegal refineries scattered around the city. This could result in the inflammation of the respiratory organs leading to an increase in pro inflammatory cytokines in individuals as stated by Ekhator *et al*. (2024)

The significant difference in the level of IL 6 in people living with HIV compared with patients' mono infected with HCV and those co-infected with HIV/HCV with a higher level among people living with HIV than in the other two groups, suggests that HCV infection may have a negative effect on the production of IL 6.The standard level of Interleukin 6 as detected by Keating *et al.* (2017) was between 0 - 12.2 pg/ml. The three groups namely people living with HIV, HCV mono infected patients and the HIV/HCV co-infected patients all had a level of IL 6 lower than the maximum prescribed standard level.

Interleukine 6 is a pleiotropic cytokine that plays a role in the regulation of the immune response, as well as the acute phase response to infection and injury (Mukhopadhyay *et al*., 2019). It has been associated with being involved in the regulation of immune cell proliferation and differentiation of CD4+ cells as stated by Mauer *et al.* (2020). The level of IL 6 in people living with HIV was significantly higher than patients' mono infected with HCV and those co-infected with HIV/HCV. This may be responsible for the higher level of CD4+ cell count among People living with HIV than in those co-infected with HIV/HCV, since IL 6 according to Mauer *et al.* (2020) is said to be responsible for the differentiation and elongation of the life span of the CD4+ cells. The mean value of the IL6 in HCV mono infected patients was higher than that found in patients co-infected with HCV/HIV. This also suggests that HCV infection may have a negative impact on the restoration of the CD4+ cells, which may be worsened by the co-infection of HIV and HCV. This study agrees with the work done by Mourtzikou *et al*. (2014) which showed a decrease in the level of Interleukin 6 in patients infected with HCV mono infection and that of Keating *et al*. (2017) among HIV/HCV co infection compared to the maximum standard level.

The finding from this study, showed a significant increase in patients' co-infected with HIV/HCV compared with the level found in people living with HIV and patients' mono infected with HCV. This finding suggests that the co-infection of both viruses has a negative impact on the IL 12 by increasing its' level, there by worsening the disease's prognosis.

The mean value for IL 12 gotten from this study in people living with HIV was higher compared with the finding documented by Essien-Baidoo *et al.* (2019). This may be due to difference in the length of time of administration of the antiviral medication and also response to the medication by the individuals. The mean level of IL 12 observed in this study among patients' mono infected with HCV, was quite higher than that reported in the work carried out by Nour el deen *et al*. (2011) and the level in HIV/HCV patients' was also higher than that reported by Chen *et al.* (2020). The high level of IL 12 as reported in this study, may be due to environmental factors such as environmental pollution and anthropogenic activities leading to the release of particulates which are capable of causing inflammation of the lungs, thereby leading to release of cytokines in the body.

From the work done by Khan and Ali (2015), the standard level of Tumour necrosis factor alpha was given to be 21.5pg/ml. In this study, the Tumour necrosis factor alpha mean level showed no significant difference across the three groups namely people living with HIV, HCV mono infected and HIV/HCV co-infected. This suggests

that the production of TNF in both HIV and HCV mono infection and their co-infection does not have any significant difference. The level of TNF in people living with HIV was recorded to be higher than that recorded by Osuji *et al.* (2018), while in patients' mono infected with HCV, the mean value in this study agrees with the level of TNF found in the work done by Pereira *et al*., (2019), although higher than the level found in the work done by R-Viso *et al*. (2010). In patients' co-infected with HIV/HCV, the mean value was found to be higher than that recorded by Chen *et al.* (2020). The increase in the TNF mean levels in the three groups above that reported in previous findings may be due to health conditions of the sampled people in addition to environmental factors

CONCLUSION

In this study, it was discovered that the levels of the interleukins studied except for IL 6 which was within the normal range, were all higher that the levels reported in previous studies which suggests that environmental factors may be responsible for the increase in the level of the interleukins in the studied groups. That environmental pollution, especially due to soot should be prevented as it has the ability to cause the release of cytokines.

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