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RESEARCH PAPER

Inverse Correlation between Combination of Antiretroviral Therapy with the level of Serum Interleukin-6, Ferritin, and Hepcidin in HIV Patients with Anemia Chronic Disease

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Abstract

Background: The purpose of this study was to determine whether the cARV therapy correlates with the serum level of IL-6, ferritin, and hepcidin in HIV infected patients with ACD.Methods: A cross-sectional study conducted in HIV patients with ACD, aged 15-65 years with exclusion criteria such as chronic renal disease, chronic liver disease, treated for anemia in the last 3 months, taking iron supplements in the last 3 months, history of blood transfusion in the last 1 year, suffering from acute infection, tuberculosis infection, malignancy, hepatitis C virus infection, acute hypersensitivity reaction and pregnancy. Blood specimen examined by high sensitivity human IL-6 In vitro ELISA, DRG Hepcidin- 25 ELISA, serum ferritin by agglutination method and CD4 cell count by flow cytometry. Results: A total of 86 HIV patients with ACD consist of 42 subjects with experienced cARV and 44 subjects with naive cARV. There are several differences between cARV naïve and experienced cARV including: Hb (g/dl) [10.8 (9.88 – 11.75) Vs. 11.60 (11.00 – 12.34) p: 0.058], serum iron (mg/dl) [50.00 (31 – 66.8) Vs. 71.40 (56.00 – 108.00) *p*: 0.121), serum ferritin (ug/dl) [630 (194 – 1101) Vs. 195.40 (128.25 – 589.47) p: 0.008], IL-6 (pg/mL) [7.20 (3.88 – 10.76) Vs. 4.03 (1.69 – 8.49) p: 0.009), serum hepcidin (ng/mL) [45.54 (16.74 – 95.31) Vs. 23.35 (6.58 – 49.99) p: 0.007] , CD4 cell count (cell/uL) [31.5 (14.25 – 90.75) Vs. 307 (168.5 – 458.75) p : < 0.001)] There is a significant inverse correlation between the cARV therapy with level of serum IL-6 (r: - 0. 285, *p*: 0.008), ferritin (r: - 0.321, *p*: 0.007) and hepcidin (r: - 0.293, *p*: 0.006). While there was a significant positive correlation between the cARV therapy with CD4 cell count (r: 0.676, *p*: 0.001). There was no correlation between the cARV therapy with serum iron levels (r: 0.176, *p*: 0.121).Conclusion: There is an inverse correlation between the cARV therapy with serum level of IL-6, ferritin, and hepcidin in HIV patients with ACD.

Keywords: *cARV, ACD, IL-6, Ferritin, Hepcidin.*

Introduction

The human immunodeficiency virus (HIV) Infection is a global health problem. People living with HIV around the world currently estimated about 36.7 million (34.0-39.8 million).[1] In Indonesia, the number of cumulative cases of HIV infection and AIDS until March 2014 were respectively 134.042 cases and 54.231 cases.[2] Anemia is a hematological disorder, often found in HIV infection at proportion approximately 37.5% (De Santis et al., 2011).[3] In Nusa Indah VCT Clinic Sanglah Bali Hospital, the prevalence of anemia in HIV infection was 29.6%.[4]Anemia in HIV infected patients can severely impact the quality and life expectancy, so that anemia is one of the biomarkers of poor outcome in patients with HIV. So far is unclear whether anemia is the result or as part of a global damage on the axis of inflammation involving various regulatory cytokines.[5] Most of the anemia in HIV infected patient is anemia of chronic disease (ACD).[6] IL-6 is the primary cytokine that plays a role in ACD pathogenesis, through translational and transcriptional induction of ferritin, and induction of hepcidin production by hepatocytes. Role of hepcidin in iron homeostasis is through inhibiting iron absorption in the duodenum and inhibiting the release of iron by macrophage, and so that the iron is not enough for the process of erythropoiesis. [7]

Combination of antiretroviral therapy (cARV) will suppress HIV replication resulting in the recovery of the immune system, which will be followed by an increase in life expectancy and quality of life of people living with HIV. cARV therapy reportedly also can correct anemia in patients with HIV infection.[8- 10] Aim of this study was to determine whether there is a correlation between cARV therapy with level of serum IL-6, ferritin, and hepcidin in HIV patients with ACD.

Methods

A cross-sectional study conducted in outpatient polyclinics Sanglah Hospital Denpasar Bali from 1 January to 1 September 2016. Sampling method was purposive consecutive in HIV patients with ACD, aged 15-65 years, with exclusion criteria such as chronic renal disease, chronic liver disease, treated for anemia in the last 3 months, taking iron supplements in the last 3 months, history of blood transfusion in the last 1 year, suffering from acute infection, tuberculosis infection, malignancy, hepatitis C virus infection, acute hypersensitivity reaction and pregnancy. HIV diagnosis is made based on the result of reactive 3 methods of anti-HIV test. The diagnose of ACD based on level of Hb < 13 g/dl in male or < 12 g/dl in female, with normochromic-normocytic morphology (MCV: 80 - 96 fl and MCH 27 - 33 pg) or hypochromic-microcytic (MCV < 80 fl and MCH < 27 pg) with level of serum iron < 50 mg/dL, total iron binding capacity (TIBC) ≤ 350 mg/dL and ferritin ≥ 30 ng/ml, with exclusion of anemia in chronic kidney disease, chronic liver disease and hyperthyroid.[11,12] This study approved by the ethics committee of udayana university - Sanglah Bali Hospital with ethical clearance No: 109 / UN.142 / R & D / 2016. All of the participants received informed consent. Blood specimen examined by high sensitivity human IL-6 In vitro ELISA, DRG Hepcidin - 25 serum ELISA, serum ferritin by agglutination method and CD4 cell count by flow cytometry.

Data research has been analyzed using SPSS 15.0 for Windows. The analysis was conducted on several variables as follows: All data were tested for normality with the Kolmogorov-Smirnov test. The normal distribution data presented by mean ± SD, while not normally distributed data are presented as the median (interquartile range).

The comparison between naïve cARV and experienced cARV was analyzed by nonparametric *Man Whitney U test*. Spearman correlation test to determine the correlation between cARV therapy with serum levels of ferritin, IL-6, hepcidin and CD4 cell counts in HIV infected patients with ACD. The level of significance (α) in this study determined the probability value (*p*) less than 0.05

Results

Characteristics

There were 86 subject HIV infected patients with ACD, 51.2% without cARV therapy (naïve cARV) and 48.8% with cARV (experienced cARV). The characteristics of the subject of HIV patients with ACD shown in Table 1.

Table 1: The characteristics of HIV subjects with ACD

Parameter	N = 86 (%)	Mean ± SD or median (IQR)
Age (years)		35 (30 - 47)
Sex		
- Male	41 (47.7)	
cARV therapy		
- *Naïve*	44 (51.2)	
- *Experienced*	42 (48.8)	
Height (Cm)		163.5 (159 - 167)
Body weight (Kg)		52.83 ± 10.35
Body mass index (Kg/M^2)		19.26 (17.57 – 21.98)
History IVDU	1 (1.16%)	
Smoking	10 (11.6)	
Alcohol	3 (3.5)	
Hemoglobin (g/dl)		11.34 (10.23 – 11.78)
- Male		11.56 10.42 – 12.70)
- Female		11.30 (10.11 – 11.60)
MCV (fl)		88.93 ± 7.63
MCH (pg)		28.15 ± 3.85
Serum iron (mg/dl) (n= 77)		56 (33 - 74)
TIBC (mg/dl) (n= 59)		232 (189 - 278)
Ferritin (ug/dl) (n= 70)		490.85 (156.75 - 1084)
Serum Creatinine (mg/dl)		0.76 (0.66 – 0,90)
Creatinine clearance (ml/mnt)		87.03 (66.49 – 109.47)
Serum Interleukin -6 (pg/mL)		5.29 (2.35 – 9.88)
Serum Hepcidin (ng/mL)		37.50 (11.15 – 60.05)
- Male		42.49 (24.31 – 83.79)
- Female		19.07 (5.93 – 52.66)
CD4 cell count (cell/uL)		133 (28.75 – 323.50)

The median CD4 cell count in HIV naïve cARV with ACD significantly lower than HIV experienced cARV with ACD. While the median serum level of IL-6, ferritin, and hepcidin in HIV naïve cARV with ACD significantly higher than HIV experienced cARV with ACD, Table 2.

Table 2: the comparison of serum hemoglobin, iron, ferritin, IL-6, hepcidin, and CD4 cell count between HIV patient cARV naïve with ACD and HIV patient cARV experienced with ACD

Variable	HIV naïve Median (IQR)	HIV experienced Median (IQR)	*P*
Hemoglobin (g/dl)	10.8 (9.88 – 11.75)	11.60 (11.00 – 12.34)	0.058
Serum Iron (mg/dl)	50.00 (31 – 66.8)	71.40 (56.00– 108.00)	0.121
Total iron binding capacity (mg/dl)	217 (180.50 -258.50)	224 (183.00 – 272.50)	0.321
Serum ferritin (ug/dl)	630 (230 – 1081)	191.80 (127.50 – 536.75)	**0.008**
Serum IL-6 (pg/ml)	7.20 (3.88 – 10.76)	4.03 (1.69– 8.49)	**0.009**
Serum hepcidin (ng/ml)	45.54 (16.74 – 95.31)	23.35 (6.58– 49.99)	**0.007**
CD4 cell count (cell/uL)	31.5 (14.25 – 90.75)	307 (168.5 – 458.75)	**< 0.001**

The correlation between cARV therapy with levels of serum IL-6, serum hepcidin and CD4 cell counts in HIV infected patients with anemia of chronic disease.

In the table 3 show cARV therapy inversely correlated to serum levels of ferritin, IL6, and hepcidin, and positively correlated with CD4 cell count. Besides, it seemed also IL-6 levels were positively associated with serum hepcidin and negatively related to CD4 cell count. While serum ferritin positively correlated to serum hepcidin and negatively correlated with CD4 cell count.

Table 3: The correlation between cARV therapy with serum levels of IL-6, hepcidin, and CD4 cell counts in HIV infected patients with ACD

Variable	Coefficient correlation	cARV	Hb	SI	TIBC	Ferritin	IL6	Hepcidin	CD4
cARV	Coefficient correlation	1.000	0.206	0.178	0.130	- 0.321	- 0.285	- 0.293	0.676
	(*p* value)	.	0.57	0.121	0.325	**0.007**	**0.008**	**0.006**	**< 0.001**
Hb	Coefficient correlation		1.000	- 0.265	- 0.035	- 0.019	- 0.138	- 0.005	0.136
	(*p* value)			**0.020**	0.792	0.877	0.205	0.966	0.211
SI	Coefficient correlation			1.000	- 0.12	- 0.038	- 0.329	- 0.147	0.147
	(*p* value)				0.931	0.759	**0.003**	0.203	0.201
TIBC	Coefficient correlation				1.000	- 0.469	- 0.245	- 0.476	0.118
	(*p* value)					**< 0.001**	0.062	**< 0.001**	0.375
Ferritin	Coefficient correlation					1.000	0.170	0.585	- 0.386
	(*p* value)						0.160	**< 0.001**	**< 0.001**
IL6	Coefficient correlation						1.000	0.541	- 0.271
	(*p* value)							**< 0.001**	**0.012**
Hepcidin	Coefficient correlation							1.000	- 0.240
	(*p* value)								**0.026**
CD4	Coefficient correlation								1.000

Discussion

cARV therapy is dramatically decreasing morbidity and mortality in HIV infection. The Effect of cARV therapy on inflammatory biomarkers until now still not clearly known.[13] In general, the levels of serum IL-6 in HIV infected patients with cARV were lower compared to those without cARV therapy.[14] Likewise as the CD4 cell count in HIV patients with anemia, Wisaksana R, (2013) found that CD4 cell count was lower in patients without cARV compared with those on cARV.[15] This is consistent with the finding in our study. The controversy appears on serum hepcidin levels where the levels of serum hepcidin were lower in HIV infected patients without cARV compared to those with cARV.[16] While Armitage, (2014) found that level of serum hepcidin in HIV infected patients with cARV was lower than without cARV therapy.[14]

In this our study found cARV therapy in HIV infected patients with anemia of chronic disease, both have a strong positive correlation with the CD4 cell count and weak negative correlation with inflammation marker (serum ferritin, IL-6, and hepcidin). It shows that the inflammation activity still persists despite immunological improvements in HIV infected patients with anemia of chronic disease, who are treated with cARV. It is probably due to HIV replication is still ongoing and gastrointestinal barrier damage as a result of microbial translocation.[18]

On the other side, we also found a weak negative correlation between serum IL-6 with hepcidin and CD4 cell counts. We also found a negative correlation between serum ferritin with hepcidin and CD4 cell count. It shows that the inflammation process have a negative correlation on the improvement of immune status, and vice versa.

While on the other hand there is a strong positive correlation between serum ferritin and IL-6 with serum hepcidin (r: 0541). This is consistent with studies in mice model, which found the role of IL-6 was the powerful induction of hepcidin production.[19] It also concurred with results of the studies on patients with ACD in some underlying chronic diseases, which found that IL-6 affects the ACD through hepcidin.[20]

The limitation of our study was not assessed the HIV-1 RNA, so the correlation between viral load and levels of serum ferritin, IL-6, hepcidin, and CD4 cell count was not measured. Besides that, the levels of serum IL-6 and hepcidin may also influence by undetectable of subclinical tuberculosis, as we known Indonesia is the region with high prevalence of tuberculosis**.**

In conclusion, cARV therapy inversely correlation with the level of serum IL-6, ferritin, and hepcidin in HIV patients with anemia of chronic disease. So it is necessary intensive applications cARV therapy while still searching for and managing the other underlying causes of ACD in HIV patients. Further research with cohort design is needed to determine the effect of inflammatory factors in HIV patients with ACD.

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