Differences of serum interleukin-6 levels in normotensive and preeclampsia women

S N Lumbanraja¹*, R Junitasari¹ and H P Pasaribu¹

¹Fetomaternal Division, Departement of Obstetrics and Gynecology, Haji Adam Malik General Hospital, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia
*Corresponding author: sarmalumbanraja@yahoo.com; snlumbanraja@gmail.com

Abstract. Red cell distribution width (RDW) has been shown higher with the increase of inflammatory activity. As CD4 count is not available in all cities in Indonesia, RDW was hoped to be the next promising marker to predict the progression of HIV infection. This study aims to find the association of RDW with CD4 count in HIV-positive reproductive women in Indonesia. It was a prospective cross sectional study enrolled 37 consented outpatients HIV-positive reproductive women in Haji Adam Malik General Hospital, Medan, Indonesia. A 10 mL blood was taken, separated into two tubes for complete blood count and CD4 count. Data were collected and analyzed with SPSS 19. Mean CD4 absolute was lowest in >14.5% RDW group but without statistical significance. By classify CD4 absolute count, CD4 absolute ≤350/mm³ showed significantly higher RDW than CD4 absolute >350/mm³ (13.98±2.10% vs 12.79±0.88%; p=0.02). No correlation was between RDW and CD4 absolute, as well as, with CD4%. In conclusion, high RDW can be a promising marker to predict the low CD4 count in HIV-positive reproductive women within antiretroviral therapy.

1. Introduction
Red cell distribution width (RDW) is a quantitative measure of variability in the size of circulating erythrocytes that are commonly used to differentiate several types of anemias.[1] Recent studies had shown that RDW could be a promising marker for inflammatory activity.[2] Moreover, RDW was known to be a strong predictor of mortality in the general population.[3]

Human immunodeficiency virus (HIV) could cause a direct cytotoxic effect on the bone marrow or indirectly via inflammation activation of cytokines to depress red blood cell count. Loss of nutrition support due to loss of appetite and side effects of ARV further complicated the loss of cell count.[4] In another side, erythropoietin compensation failed due to molecular mimicry with HIV-1 p17 protein.[5] The decrease of red blood cell count caused red cell distribution width to increase due to impaired erythropoiesis and abnormal red blood cell survival. Few studies have shown the high level of RDW reflects the progression of infection or inflammation.[6] As RDW varies among populations, this study aims to find RDW association with CD4 in HIV patients in Indonesia.

2. Methods
It was a prospective cross sectional study conducted in Haji Adam Malik General Hospital as the tertiary hospital in Sumatera, Indonesia, in July to August 2016. All reproductive women admitted to
this hospital was informed about the study and consented to participate. We included only the HIV-positive reproductive women within regular antiretroviral therapy to find how RDW could predict HIV progression in a specific population. Subjects were to the exclusion if they had trauma-related injury, malignancy, or has just discharged from the hospital within the previous seven days.

All consented subjects were asked permission for blood sampling in vena cubiti. Two tubes of each 5 mL blood were taken, one for complete blood count, and one for the CD4 count. RDW was measured as a part of the automated complete blood count device with reference range of 11.5 to 14.5% in our institution. CD4 was analyzed using flow cytometry and describe as CD4% and CD4 absolute. In order to compare the RDW between HIV-positive and HIV-negative subjects, the same number of control subjects were to the inclusion. Healthy subjects were those who enrolled in blood transfusion programme that undergoes HIV screening before donor.

Continuous variables were as means ± standard deviations (SD) and categorical variables were as percentages. Data were tabulated and processed in SPSS 19.0 (Chicago, IL). Correlation between RDW, CD4%, and CD4 absolute or RDW, WBC, and ESR was by using Pearson correlation. Difference between the length of ARV consumption and RDW were done using t-independent test. This study used p<0.05 as the significance cut off point.

3. Results
This study enrolled 37 consecutive HIV-positive reproductive women on antiretroviral therapy. Mean age of subjects in this study was 34.14±4.16 years old. Mean RDW in HIV-positive reproductive women was 13.08±1.35%, higher than in HIV-negative reproductive women with 11.43±1.06%. However, There was no significant difference of RDW between HIV-positive and HIV-negative reproductive women (p=0.621). Regarding of the length of antiretroviral consumption, subjects with more than three years therapy had insignificant higher RDW (13.66±1.96%) than those with less than three years therapy (12.89±1.07%) (p=0.205). There was no difference of RDW regarding of subjects age (p=0.417) (Table 1).

Table 1. Mean distribution of RDW regarding of HIV status, length of antiretroviral therapy, and age.

| Category                          | RDW          | p     |
|-----------------------------------|--------------|-------|
| HIV status                        |              |       |
| HIV-positive                      | 13.08 ± 1.35 |       |
| HIV-negative                      | 11.43 ± 1.06 |       |
| Length of antiretroviral therapy  |              |       |
| ≤3 years                          | 12.89 ± 1.07 | p=0.205|
| >3 years                          | 13.66 ± 1.96 |       |
| Age                               |              |       |
| ≤34 years old                     | 13.06 ± 1.48 | p=0.417|
| >34 years old                     | 13.10 ± 1.27 |       |
| CD4                               |              |       |
| ≤350/mm³                          | 13.98 ± 2.10 | P=0.02 |
| >350/mm³                          | 12.79 ± 0.88 |       |

As 11.5-14.5% was the reference range of RDW in this study, RDW was classified based on that reference. Mean CD4% and CD4 absolute were highest in normal RDW group with 23.37 ± 6.55% and 515.50 ± 230.71, respectively (Table 2). Mean CD4 absolute was lowest in >14.5% RDW group, but statistically, there was no correlation were found between RDW and CD4 absolute, as well as, with CD4%, white blood cell (WBC), and erythrocyte sedimentation rate (ESR) (Table 3). However, by classify CD4 absolute count, CD4 absolute ≤350/mm³ showed significantly higher RDW than CD4 absolute >350/mm³ (13.98 ± 2.10% vs 12.79 ± 0.88%; p=0.02) (Table 1).
4. Discussion
Regardless of its cardiovascular risk, HIV infection associated with a large role of cytokine activation.[7] RDW has been as markers of inflammation that predict the prognosis across several types of diseases.[8] Proinflammatory cytokines like interleukin 1 (IL-1), IL-6, IL-10, and TNF-alpha are the main cytokines to be the reason for elevated RDW levels.[9]

In HIV-positive subject to antiretroviral therapy as well as not on antiretroviral therapy were showed to have a significant increase in RDW compared to the control subjects. Moreover, MCH and MCHC decreased, as usual, suggested macrocytic hypochromic anemia.[10] The inflammatory state in HIV led to impaired erythrocyte maturation and anisocytosis, increasing RDW.[11]

In this study, using 350/mm$^3$ as a cut-off point to classify CD4 absolute count, CD4 absolute ≤350/mm$^3$ showed significantly higher RDW than CD4 absolute >350/mm$^3$ (13.98 ± 2.10% vs 12.79 ± 0.88%; p=0.02). However, statistical analysis with Pearson correlation showed no correlation between CD4% or CD4 absolute and RDW. It remarked the role of RDW (>13.98%) to predict only the HIV-positive subjects with the low CD4 count but not to predict linearly how CD4 absolute count would be.

Gallego et al. showed that mean RDW in HIV-positive subjects was 13.7%. Comparing with control, the top percentile of RDW was associated with HIV-positive infection (OR 1.6; 95% CI 1.0-2.4; p = 0.02) and detectable viral load (OR 1.5; 95% CI, 1.01-2.4; p = 0.04). Adediran et al. proved an increase of RDW in patients with HIV compared to controls (p=0.028).[13] This study showed the lower RDW in HIV-positive subjects with 13.08 ± 1.35%. It could be due to the low sample size or the analyzing the specific population in reproductive women with antiretroviral therapy, differ with general population sample in the previous study. This study also showed in contrast results that no significant difference of RDW between HIV-positive and HIV-negative subjects. Puerta et al. (2010) showed the similar mean of RDW with this study, 13.07% (7.7-33.6%), with the same sample, HIV-infected outpatients in antiretroviral therapy. However, they proved that high RDW (>14.1%) associated with HIV-positive infection (p=0.02).[14]

Recent studies have shown that zidovudine therapy also associated with high RDW percentage. Rivas (2005) has previously shown that zidovudine therapy was associated with increase RDW, suggested as the markers of early adherence to zidovudine therapy.[15] However, Adediran study, group with zidovudine therapy or non-zidovudine therapy showed no significant difference in RDW scoring.[13] In this study, no subject was in zidovudine therapy. Therefore, no bias of RDW due to zidovudine therapy can occur.

This study has some limitations. Although it was analyzed HIV-positive reproductive women in antiretroviral therapy, this study did not differ the antiretroviral regimen received by the subjects. In another hand, the larger sample size was important for further study.

5. Conclusion
High RDW can be a promising marker to predict the low CD4 count in HIV-positive reproductive women within antiretroviral therapy.

### Table 2. Distribution of CD4% and CD4 absolute based on RDW classification.

| RDW    | N  | CD4%    | CD4 Absolute |
|--------|----|---------|--------------|
| <11.5  | 2  | 14.50±14.85 | 412.50±528.21 |
| 11.5-14.5 | 30 | 23.37±6.55   | 515.50±230.71 |
| >14.5  | 5  | 18.00±14.83  | 357.20±352.16 |
| Total  | 37 | 22.16±8.48   | 488.54±260.25 |

### Table 3. Correlation between RDW, CD4%, CD4 absolute, WBC, and ESR.

| Correlation | CD4%    | CD4 Absolute | WBC | ESR |
|-------------|---------|--------------|-----|-----|
| RDW         | r=-0.112| r=-0.155     | r= 0.140 | r= 0.810 |
| p=0.508     | p=0.360 | p=0.408      | p=0.634 |
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