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Original Article

A comparison of mean corpuscular volume (MCV) between thalassemia-carrier and non-thalassemia-carrier pregnant women receiving highly active antiretroviral therapy (HAART)

Yada Thongyou, Fuanglada Tongprasert*

Department of Obstetrics and Gynecology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

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ABSTRACT

Objective: HIV-infected treatment with antiretroviral drugs is one of the common causes of macrocytosis. In patients receiving highly active antiretroviral therapy (HAART), the mean corpuscular volume (MCV) can be shifted from microcytic to normocytic or macrocytic after treatment and significantly affected the thalassemia screening. This study aimed to compare MCV between thalassemia-carrier and non-thalassemia-carrier antiretroviral drug-naïve, HIV-infected, pregnant women receiving HAART. The results will support the couples at risk identification in prenatal control of severe thalassemia disease.
Materials and methods: A retrospective cohort study was conducted in antiretroviral drug-naïve, HIV-infected, pregnant women who received HAART between January 2008 and December 2015 in Maharaj Nakorn Chiang Mai Hospital, Chiang Mai, Thailand. Changes in MCV were compared between the thalassemia and non-thalassemia carriers.
Results: Of 74 pregnant women who were exposed to HAART for at least 4 weeks, increased MCV levels were significantly greater in the non-thalassemia carriers group (n = 58) than in the thalassemia-carrier group (n = 16) (16.60 ± 12.55 fL and 15.61 ± 9.67 fL, respectively; $p < 0.001$). Pre-HAART exposure, sensitivity of MCV was 83.3% for thalassemia carriers screening using MCV <80 fL. Post-HAART exposure, sensitivity of MCV was 33.3%, and the false negative rate was 66.7%.
Conclusion: Post-HAART exposure, MCV increased substantially in both the thalassemia and non-thalassemia carriers. Using MCV <80 fL as the cutoff for diagnosing thalassemia, false negative results were observed in two thirds of the thalassemia carriers who were exposed to HAART for at least 4 weeks; therefore, the screening test should be interpreted with caution.

Introduction

Severe thalassemia is a common hereditary disease in Thailand and Southeast Asia, and should be prenatally diagnosed as the first step in a control strategy; a blood test is used to identify the carriers and couples at risk of having fetuses with severe thalassemia [1]. In Thai pregnant women, the prevalence of common thalassemia carriers including α-thalassemia-1 carriers, β-thalassemia carriers and hemoglobin E carriers were 6.6%, 3.7% and 11.6%, respectively [2]. At the first visit of antenatal care, mean corpuscular volume (MCV) is an effective, and widely used, screening test thalassemia carriers [3]. In common thalassemia carriers (α-thalassemia-1 carriers, β-thalassemia carriers and hemoglobin E carriers), microcytosis is typically identified with MCV ranges between 70 and 75 fL, 60–75 fL and 75–85 fL, respectively [4]. However, some medical conditions or certain medications can affect MCV [5]. Treating HIV patient with antiretroviral drugs – particularly nucleoside reverse transcriptase inhibitors (NRTIs), such as zidovudine, stavudine and lamivudine – is a common cause of macrocytosis. In thalassemia carriers receiving highly active antiretroviral therapy (HAART), MCV shifted from microcytic to normocytic after 6–12 months of treatment, significantly affecting thalassemia-screening results [6]. However, changes in MCV have not been studied during pregnancy, a time when thalassemia screening is usually carried out for prenatal control. This study aimed to compare MCV values between thalassemia and non-

* Corresponding author. Department of Obstetrics and Gynecology, Faculty of Medicine, Chiang Mai University, Chiang Mai, 50200, Thailand. Fax: +66 53 936112.
E-mail address: fuanglada.t@cmu.ac.th (F. Tongprasert).

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thalassemia carriers among antiretroviral drug-naïve, HIV-infected, pregnant women receiving HAART.

Materials and methods

A retrospective cohort study was conducted at Maharaj Nakorn Chiang Mai Hospital, Department of Obstetrics and Gynecology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand, with approval of the Research Ethics Committee, using a database of HIV-infected pregnant women who received antenatal care at the hospital between January 2008 and December 2015. Eligibility criteria included: 1) antiretroviral drug naïve, HIV-infected, pregnant women; 2) receiving at least 4 weeks of HAART during pregnancy; 3) having been screened and diagnosed for thalassemia carrier status; 4) having laboratory result of MCV in the first trimester or before HAART exposure and 5) having at least one laboratory result of MCV obtained at a follow-up visit (4, 8, 12, 16, 20 or 24 weeks after HAART exposure). Exclusion criteria included: 1) multiple pregnancies; 2) certain medical conditions, including heart disease, liver disease, alcoholism or chronic diseases; 3) iron deficiency anemia or anemic diseases receiving at least 200 mg of elementary iron or blood transfusions and 4) failure to adhere to HAART. The sample size was calculated based on MCV changes of at least 10 fL among the thalassemia and non-thalassemia carriers receiving HAART therapy in a previous study [6]. Sixteen pregnant women were needed in each group for a 0.05 two-sided type 1 error with 0.9 powers.

The MCV value was analyzed with an automated complete blood count (CBC) machine (Coulter STKS analyzer; Beckman, Brea, CA, USA). Thalassemia carriers were defined as those pregnant woman diagnosed as either β-thalassemia carriers, HbE carriers, or α-thalassemia-1 carriers. The hemoglobin A2 (HbA2) level, measured by microcolumn chromatography, or hemoglobin typing, based on high performance lipid chromatography (HPLC), were used to detect β-thalassemia carriers and HbE carriers. The levels of HbA2 in β-thalassemia carriers, HbE carriers, and homozygous HbE carriers were 4–9%, >10%, and >80%, respectively. The α-thalassemia-1 carriers were diagnosed by a positive polymerase chain reaction (PCR) for the α-thalassemia-1 gene (Southeast Asia [SEA] deletion and THAI deletion) [1]. HAART was defined by at least three antiretroviral drug combinations of two NRTIs (e.g., zidovudine, lamivudine, stavudine, tenofovir, emtricitabine) plus either one NNRTI (e.g., nevirapine, efavirenz) or one PI (e.g., lopinavir/ritonavir, atazanavir, indinavir, darunavir).

Patient demographic data, CD4 cell counts before and after HAART exposure, HIV RNA viral load before delivery, gestational age at the beginning of HAART, duration of HAART regimen, type of HAART (zidovudine-based or non-zidovudine-based regimen), medical conditions, obstetric complications, any treatment for anemia (such as iron supplementation or blood transfusion), and pregnancy outcomes were obtained from the database and medical records.

Statistical analysis

All statistical analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, IL). The demographic characteristics were described and compared between the thalassemia-carrier and non-thalassemia-carrier groups by the independent samples *t*-test, Mann–Whitney U test, Chi-square test and Fisher's exact test. The categorical data were presented as frequencies (%) and the continuous data were presented as means and standard deviations (SD) or medians and interquartile ranges (IQR). MCV values were compared between both groups using the Chi-square test and Fisher's exact test for categorical variables and the independent samples *t*-test for continuous variables. $P < 0.05$ was considered statistically significant.

Results

Over the 8-year period studied, 78 antiretroviral drug naïve, HIV-infected, pregnant women receiving HAART were eligible for the study. Four cases were excluded because of iron deficiency anemia (3 cases) or β-thalassemia/HbE disease with severe anemia (1 case). Of the remaining 74 cases, 58 cases (78.4%) were non-thalassemia carriers and 16 cases (21.6%) were diagnosed as thalassemia carriers (3 cases of α-thalassemia-1 carriers, 1 case of Hb constant spring carrier, 3 cases of β-thalassemia carriers, and 9 cases of HbE carriers).

The baseline characteristics and pregnancy outcomes of the study population are presented in Table 1. Maternal age, parity, body mass index, CD4 cell counts, HIV RNA viral load, gestational age at the beginning of HAART, duration of HAART regimen, type of HAART, medical conditions, obstetric complications, gestational age at delivery, route of delivery, and birth weight were not significantly different between the thalassemia-carrier and non-thalassemia-carrier groups.

Changes in MCV values in the thalassemia-carrier and non-thalassemia-carrier groups are shown in Table 2. In the non-thalassemia-carrier group, the average MCV value was 86.59 ± 7.47 fL before initiating HAART and increased to macrocytic levels (MCV $\geq$ 100 fL) after 1–6 months of receiving HAART. In the thalassemia-carrier group, the average MCV value was 78.98 ± 8.82 fL before initiating HAART and increased to normocytic levels (MCV 80–100 fL), with a maximum rise of 23.50 fL, after 6 months of receiving HAART, as shown in Fig. 1.

Among the thalassemia carriers, the prevalence of microcytosis (MCV < 80 fL) before initiating HAART was 56.25% (9 cases). The pre-exposure MCV sensitivity and specificity for detecting α-thalassemia-1 carriers and β-thalassemia carriers was 83.3% and 81.0%, respectively. Post-HAART exposure, the sensitivity, specificity and false negative rate of MCV was 33.3%, 98.3% and 66.7%, respectively.

Discussion

Pregnancies complicated with HIV infection are principally managed with highly active antiretroviral therapy (HAART), which lowers the rate of mother-to-child transmission (MTCT) to between 0.1 and 1% [7]. All HIV-pregnant women should start HAART as early as possible, regardless of CD4 cell counts or HIV viral load, for adequate viral suppression by the time of delivery [8]. Antiretroviral drug combinations of two nucleoside reverse transcriptase inhibitors (NRTIs), plus either a non-nucleoside reverse transcriptase inhibitor (NNRTI), a protease inhibitor (PI) or an integrase inhibitor, are the preferred HAART regimens during the antepartum period [9].

Despite their high efficacy, antiretroviral drugs, especially NRTIs, have a significantly negative effect on hematologic parameters [10]. Research has reported lower hemoglobin levels (Hb), red blood cell counts (RBC) and white blood cell counts (WBC), along with higher mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and hemoglobin A2 (HbA2) [10,11]. In HIV patients receiving a zidovudine-based HAART regimen, MCV has been shown to increase significantly after one month of HAART exposure; the development of macrocytosis could be used as an adherence indicator to reflect the treatment effectiveness [12,13]. Macrocytosis has also been reported in patients taking other NRTIs, such as stavudine and lamivudine, but to a lesser degree [12,14,15]. In brief, drug-induced impairment of DNA synthesis is expected to be a mechanism of megaloblastic change [16]. By functioning as

Table 1
Baseline characteristics of HIV-infected pregnant women receiving HAART.

Baseline characteristics	Total (74 cases)	Thalassemia carriers (16 cases)	Non-thalassemia carriers (58 cases)	*p*-value
Maternal age (yrs, mean ± SD)	27.9 ± 6.4	26.9 ± 7.2	28.1 ± 6.1	0.491
$CD4^+$ pre-HAART exposure (cells/mm^3, median and 25th, 75th percentile)	404.0 (209.5, 501.5)	418.0 (167.5, 533.5)	402.0 (223.0, 449.5)	0.849
$CD4^+$ before delivery (cells/mm^3, median and 25th, 75th percentile)	462.0 (350.0, 621.0)	433.0 (356.5, 643.0)	471.0 (330.5, 621.0)	0.865
HIV RNA before delivery (copies/mL, median and 25th, 75th percentile)	40.0 (40.0, 160.0)	40.0 (40.0, 400.0)	40.0 (40.0, 97.0)	0.881
GA at the beginning of HAART (wks, median and 25th, 75th percentile)	17.0 (14.0, 22.0)	16.0 (14.0, 25.0)	15.0 (13.0, 20.0)	0.802
Duration of HAART (wks, median and 25th, 75th percentile)	20.0 (14.8, 24.0)	20.0 (14.5, 24.0)	23.0 (18.0, 25.0)	0.414
GA at delivery (wks, median and 25th, 75th percentile)	38.0 (37.0, 39.0)	39.0 (35.5, 39.5)	38.0 (38.0, 39.0)	0.106
Birth weight (g, mean ± SD)	2847.2 ± 418.6	2856.0 ± 530.0	2844.0 ± 387.0	0.917
Route of delivery (%)				
• ND	63.5	50.0	67.2	0.541
• Elective CS	13.5	18.8	12.1	
• Emergency CS	21.6	31.3	19.0	
• VE, FE, BA	1.4	0.0	1.7	

yrs, years; SD, standard deviation; cells/mm3, cells per cubic millimeter; copies/mL, copies per milliliter; GA, gestational age; wks, weeks; g, gram; ND, normal delivery; CS, cesarean section; VE, vacuum extraction; FE, forceps extraction; BA, breech assisting delivery.

Table 2
Comparisons of changes in MCV values between thalassemia- and non-thalassemia-carrier pregnant women receiving HAART.

Variables	Total (74 cases)	Thalassemia carriers (16 cases)	Non-thalassemia carriers (58 cases)	*p*-value
MCV Pre-HAART exposure	85.0 ± 8.3	79.0 ± 8.8	86.6 ± 7.5	<0.001
MCV Post-HAART exposure	101.3 ± 13.8	94.6 ± 14.7	103.2 ± 13.1	<0.001
Changes in MCV[a]	16.4 ± 11.9	15.6 ± 9.7	16.6 ± 12.6	<0.001

MCV, mean corpuscular volume.
[a] Changes in MCV calculated by post-HAART exposure MCV minus pre-HAART exposure MCV.

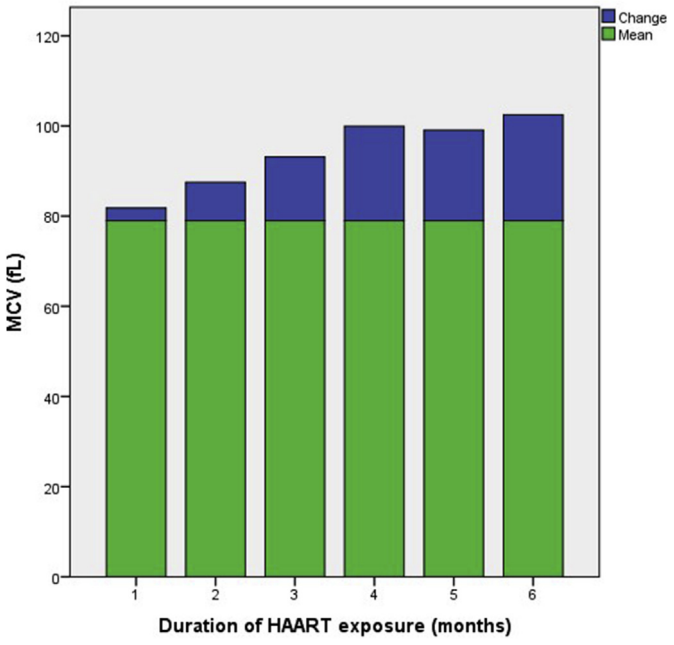

Fig. 1. Changes of MCV values in thalassemia-carrier pregnant women during each month of HAART exposure.

competitive inhibitors, NRTIs are nucleoside analogs of natural cytidine, guanosine, thymidine or adenosine, which are needed to synthesize DNA; therefore NRTIs inhibit the activity of reverse transcriptase. The slowing of DNA synthesis causes immature nuclear maturation, while normally mature cytoplasm leads to a large, immature nucleus relative to a normal appearance cytoplasm, is known as the nuclear-cytoplasmic dissociation [17].

In Thailand, MCV is usually used to screen for inherited thalassemia disease, especially during antenatal care in order to control prenatal thalassemia [1]. Although MCV has been proven an effective screening test, many conditions that may cause larger red blood cells can lead to false negatives, and need to be taken into account [5]. Among 14 HIV-infected male and female thalassemia-inherited adult patients, mean MCV shifted from microcytic (71.81 fL) to normocytic (80–100 fL) levels without severe anemia at months 6 and 12 of HAART exposure [6]. In our study of 16 HIV-infected, thalassemia-inherited, pregnant women, mean MCV gradually rose following initiation of HAART, by 2.83 fL after one month of HAART exposure and up to 23.50 fL after six months of HAART exposure. Even in different populations, similar results were identified in this study of healthy HIV-infected women, with baseline CD4 cell counts of 355 cells/mm^3, compared to a previous study of HIV-infected adults, with baseline CD4 cell counts of 120 cells/mm^3 [6].

In this study, the pre-HAART exposure MCV sensitivity for detecting α-thalassemia-1 carriers and β-thalassemia carriers was 83.3%, while post-HAART exposure MCV sensitivity dropped to 33.3%; this compared to 92.9% sensitivity of MCV from previous research in non-HAART exposure population [3]. In other words, approximately two-thirds of the carriers who had abnormal MCV screening tests for thalassemia (MCV < 80 fL) gave false negative results after at least one month of HAART exposure. Seriously, the false negative MCV screening test will lead to miss the couple at risk for having severe thalassemia disease in their fetuses. The alternative high efficacy screening test e.g. the osmotic fragility test (OFT) may be considered in this population [18]. The molecular analysis of thalassemia gene mutation or deletion using various techniques may be required to confirm a diagnosis in highly suspicious cases.

Although an adequate amount of thalassemia carriers was recruited into our study, the small number of α-thalassemia-1 carriers and β-thalassemia carriers may have limited the power to evaluate the accuracy of MCV. This study was also limited by its retrospective nature, as we were not able to control for all of the confounders that can affect the size of red blood cells. We tried to

reduce this weakness by excluding patients with any diseases likely to affect red blood cell size or those taking any medications other than HAART. Our study assessed MCV values from the first month of HAART exposure and every month afterwards; this was earlier and more frequently than previous research, a strength of our study.

In conclusion, pregnant women receiving HAART regimens were associated with substantial increases in MCV, in both thalassemia and non-thalassemia carriers. Using MCV <80 fL as the cutoff for diagnosing thalassemia carriers, false negative results were observed in two-thirds of the thalassemia carriers from as early as 4 weeks after HAART exposure; thus the MCV screening test should be interpreted with caution in pregnant women receiving HAART. History taking about partner's HIV status and treatment is also necessary for the couples at risk identification in prenatal control of severe thalassemia disease. Further investigations or diagnostic tests should be prompt to evaluate in these cases of uncertainty.

Conflicts of interest statement

The authors have no conflicts of interest relevant to this article.

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