RESEARCH PAPER

Hematological Alterations and Thymic Function in Newborns of HIV-Infected Mothers Receiving Antiretroviral Drugs

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Objectives: To investigate the effects of antiretroviral (ARV) drugs on hematological parameters and thymic function in HIV-uninfected newborns of HIV-infected mothers.

Study design: Cross sectional study.

Setting: Chiang-Mai University Hospital, Chiang-Mai, Thailand.

Participants/Patients: 49 HIV-uninfected and 26 HIV-infected pregnancies.

Methods: Cord blood samples of newborns from HIV-uninfected and HIV-infected mothers were collected. Hematological parameters were measured using automatic blood cell count. T-cell receptor excision circles (TRECs) levels in cord blood mononuclear cells (CBMCs), $CD4^+$ and $CD8^+$ T-cells were quantified using real-time PCR.

Main Outcome Measures: Hemotological parameters and thymic function.

Results: Newborn of HIV-infected mother tended to have lower mean levels of hemoglobin than those of HIV-uninfected mother (137 ± 22 *vs* 146 ± 17 g/L, $P = 0.05$). Furthermore, mean of red blood cell (RBC) counts and hematocrit and median of TRECs in $CD4^+$ T-cells in the newborns of the former were significantly lower than those of the latter [3.6 ± 0.7 *vs* 4.8 ± 0.6 x 10^{12} cells/L, $P <0.001$; 0.40 ± 0.07 *vs* 0.46 ± 0.05 L/L, $P <0.001$ and 0.53 (IQR: 0.03-5.76) *vs* 13.20 (IQR: 2.77-27.51) x 10^{-3} pg/μL, $P = 0.02$, respectively].

Conclusion: ARV drugs altered hematological parameters and thymic function (TRECs $CD4^+$ T-cells) in HIV-uninfected newborns of HIV-infected mothers.

Key Words: *Adverse effects, Antiretroviral drugs, Hematology, HIV, Newborn, Thymic function .*

Almost half of the estimated 40 million people living with HIV are women of childbearing age [1]. The risk of these women to transmit HIV to their infants is 15-25% when no precautions are taken [2]. The HIV-mother-to-child transmission (MTCT) rate has dramatically reduced to be less than 2% with antiretroviral (ARV) prophylaxis during pregnancy and labor as well as to the infant [3, 4]. The previous studies showed that Zidovudine (ZDV) which is a potent inhibitor of bone marrow function is associated with hematological abnormalities not only in mothers, but also in newborns, because this drug can cross the placental barrier and negatively affect fetal erythropoiesis [5-8]. Moreover, ZDV-based HAART is commonly associated with a greater negative impact on hematological parameters than ZDV-free regimens [9]. The adverse hematological effects of ARV drugs have been reported in HIV-uninfected infants, especially in their early life [10]. The frequently adverse hematological effects found are anemia, neutropenia, lymphocytopenia and thrombocytopenia [7, 11-13].

The thymus is a primary source of naïve T-cells and plays a key role in establishing and maintaining a peripheral T-cell pool [14]. Thymus reaches its maximum volume by one year of age [15]. A production of naïve T-cells by the thymus can be quantified by measuring T-cell receptor excision circles (TRECs), a DNA fragment formed during T-cell development. These DNA fragments do not replicate during mitosis and are thus diluted during cell division [16]. Previous studies demonstrated that both HIV-proteins and some antiretroviral drugs inhibited progenitor cells and thymic functions, as indicated by the frequency of TRECs [17-19]. However, an evaluation of hematological and immunological toxicity in newborn exposed to maternal ARV drugs administered during pregnancy has been limited. The aims of this study were to measure and compare hematological parameters and TRECs levels in HIV-uninfected newborn of HIV-infected mother receiving ARV drugs for prevention of HIV-MTCT with those of normal control newborn.

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METHODS

This study was conducted at Chiang-Mai University Hospital, Chiang-Mai, Thailand. The protocol was approved by the Faculty of Medicine Ethics Committee, Chiang-Mai University, Chiang-Mai, Thailand. All pregnant women participating in this study had signed a written informed consent. To obtain the subjects, the exclusion criteria for the study were set as follow: women with twin or multiple births, infected with other micro-organisms, and use of psychopharmaceutical drugs, illicit drugs, alcohol and tobacco during gestation. From March to December 2011, 26 HIV-infected and 49 HIV-uninfected pregnant women were enrolled. These HIV-infected women received ARV drugs [ZDV plus Lamivudine (3TC) and Lopinavir/Ritonavir (LPV/r)] during pregnancy and labor every 12 hours with adding of ZDV every 3 hours during labor and delivered vaginally or elective caesarean section. The following data were collected from all women: age, gestational age at delivery and mode of delivery. For the HIV-1 infected women, the following additional data were collected: antiretroviral prophylaxis (type and timing), $CD4^+$ T-cell counts (cells/μL) during pregnancy and plasma HIV-1 RNA viral load measured a week before delivery (log_{10} copies/mL). All women in our study were given iron and folate supplementation as recommended by the Thai National Guidelines for Pregnancies [20]. Diagnosis for HIV-1 infection in infants born to HIV-1 infected mothers was performed at one and four months of age using DNA PCR (Amplicor HIV-1 DNA assay version 1.5, Roche Molecular Systems Inc., USA).

Isolation of cord blood mononuclear cells (CBMCs): Cord blood samples were drawn from clamped umbilical vein within 5-10 minutes after delivery into ethylenediamine tetraacetic acid anticoagulation (EDTA) tubes (BD Vacutainer, Franklin Lakes, NJ, USA). The sample tubes were then shipped to the hematology laboratory, Faculty of Associated Medical Sciences, Chiang-Mai University within 3 hours. Upon arrival, hematological parameters were measured using an automated blood counter (Sysmex KX-21; Sysmex Corporation, Kobe, Japan). Cord blood mononuclear cells (CBMCs) were isolated using Ficoll-Hypaque gradient (IsoPrep, Robbins Scientific, Sunnyvale, CA, USA). Cells were then aliquoted and stored in liquid nitrogen until used.

Separation of $CD4^+$ and $CD8^+$ T-cells: It was performed from CBMCs of the 15 HIV-uninfected newborns of HIV-infected mothers and only 12 HIV-uninfected newborns of HIV-uninfected mothers. Frozen CBMCs were thawed and washed twice in cold phosphate-buffered saline solution. $CD4^+$ and $CD8^+$ T-cells were separated using a magnetic cell separator (EasySep, STEMCELL Technologies, USA) according to manufacturers' instructions. The separated $CD4^+$ and $CD8^+$ T-cells cells were count on hemacytometer under light microscope using Turk's solution.

DNA Preparation and Quantification of TRECs

DNA was extracted from 1.5×10^6 cells of CBMCs, separated $CD4^+$and $CD8^+$ T-cells using the NucleoSpin kit (Macherey-Nagel, KG., Duren, Germany) according to manufacturers' instructions and was stored at -20°C until used. TRECs analysis was performed by quantitative real-time PCR as described by Ometto, *et al*. [21] with slightly modification. The DNA amplification was carried out in a 25 μL reaction mixture containing 5 μL DNA sample or sterile distilled water as a no template control, 1×real-time PCR Master Mix (Thermo Scientific ABsolute^{TM} QPCR ROX Mix, Surrey, UK), 400 nM each primer (forward, 5'-CACATCCCTTTCAACCATGCT-3'; reverse, 5'-GCCAGCTGCAGGGTTTAGG-3' : GenBank sequence accession number DQ858179.1) and 200 nM of the fluorogenic probe (5'-ACACCTCTGGTTTTT GTAAAGG TGCCCAC T-3') conjugated with FAM (6-carboxy-fluorescein) at the 5'-end, and TAMRA (6-carboxy-tetramethilrhodamine) at the 3'-end. The PCR primers and the fluorogenic probe were specifically designed for the detection of human TRECs. The amplification was performed in a Rotor-Gene 6000™ (Corbett Research; Mortlake, New South Wales, Australia). The mixture was preheated at 95°C for 15 min, followed by 50 cycles at 95°C for 15 sec and 60°C for 1 min. A cycle threshold (C_T) is defined as the PCR cycle at which an increase in the fluorescence above the baseline signal is first detected. The C_T value is inversely related to the copy number of the target sequence. TRECs concentrations were calculated from a standard curve of a plasmid clone containing TRECs which run in parallel with the test. All samples and TRECs plasmid were run in duplicate. TRECs level in CBMCs was presented as concentration of TRECs per 1.5×10^6 CBMCs while those in $CD4^+$ and $CD8^+$ T-cell was presented as concentration of TRECs per cell.

Statistical analysis: Statistical analyses were performed using SPSS software package (Statistical Package for the Social Sciences 11.0, Chicago, IL, USA). Characteristics and hematological parameters were compared between two groups of newborns using independent samples *t* test and Fisher's exact test while levels of TRECs between the two groups were compared using Mann-Whitney test. The level of significance for all analyses was set at 0.05.

RESULTS

The clinical data of participants are shown in ***Table* I**. Mean of maternal ages and gestational ages at delivery

TABLE I CHARACTERISTICS OF HIV-INFECTED AND UNINFECTED MOTHERS AND THEIR NEWBORNS

Characteristics	*HIV-infected mother (n = 26)*	*HIV-uninfected mother (n = 49)*	*P-value*
Age at delivery (y) [mean±SD (range)]	30 ± 7 (17-42)	27 ± 6 (15-42)	0.08
Gestational age at delivery (wks)	38 ± 2 (33-40)	38 ± 1 (34-41)	0.58
Gestational age at ARV prophylaxis initiation (wks)	21 ± 5 (14-27)	Not Relevant	
$CD4^+$ T-cell count during pregnancy (cells/mL)	517 ± 188 (186-859)	Not Relevant	
HIV RNA load measured at one week before delivery (copies/mL)	<40	Not Relevant	
Mode of delivery; Vaginal *vs* Caesarean	17 : 9	39 : 10	0.24
Gender of newborn, Male: Female	18 : 8	24 : 25	0.08
Birth weight of newborn (g)	2873 ± 461 (2050-3910)	3029 ± 412 (2250-3950)	0.18

were similar between HIV-infected and uninfected women. Most of HIV-infected and uninfected women delivered vaginally. The HIV RNA viral loads measured at one week before delivery of HIV-infected women were less than 40 copies/mL and none of all newborns born to HIV-1 infected mothers had HIV-infection.

Mean levels of white blood cell (WBC) counts, absolute neutrophil counts, absolute lymphocyte counts and platelet counts in newborns of HIV-infected and uninfected mothers did not differ significantly (***Table* II)**. Means of red blood cell (RBC) counts and hematocrit in newborns of HIV-infected mothers were significantly lower. On the other hand, newborns of HIV-infected mothers showed higher mean levels of red cell indices than those of HIV-uninfected mothers (***Table* II**).

No significant difference in median of TRECs levels in CBMCs (***Fig.* 1a**) and in $CD8^+$ T-cell (***Fig.* 1b**) was found between newborns of HIV-infected mothers and uninfected mothers. However, TRECs levels in $CD4^+$ T-cell (***Fig.* 1c**) in newborns of HIV-infected mothers were significantly lower than those of HIV-uninfected mothers.

DISCUSSION

The current study showed that ARV drugs (ZDV plus 3TC and LPV/r) administered to HIV-infected mother for prevention of HIV-MTCT altered the hematological parameters of newborns. Furthermore, the thymic function of these newborns was also impaired as indicated in the decrease of TRECs $CD4^+$ T-cell. The previous study showed that maternal derived HIV-proteins diffusing across the placental barrier during pregnancy could reduce thymic function [19, 22]. In addition, both HIV-proteins and ARV drugs are known to inhibit progenitor cell function [17, 18]. However, in present study, the effects of HIV-proteins on thymic function might be less than those of ARV drugs since maternal viral loads in all HIV-infected mothers measured at one week before delivery were less than 40 copies/mL.

TABLE II HEMATOLOGICAL PARAMETERS OF NEWBORNS OF HIV-INFECTED AND UNINFECTED MOTHERS

Hematological parameters	*Newborn of HIV-infected mother (n = 26)*	*Newborn of HIV-uninfected mother (n = 49)*	*P Value*
WBC (x 10^9 cells/L)	13.0 ± 5.0 (3.5-24.3)	14.6 ± 5.6 (5.3-35.1)	0.24
Absolute neutrophils (x 10^9 cells/L)	7.4 ± 2.6 (2.4-12.3)	6.5 ± 2.7 (0.8-11.5)	0.27
Absolute lymphocytes (x 10^9 cells/L)	4.8 ± 2.9 (2.1-12.8)	5.8 ± 3.1 (2.8-21.2)	0.19
RBC (x 10^{12} cells/L)	3.6 ± 0.7 (1.7-4.9)	4.8 ± 0.6 (3.7-6.2)	<0.001
Hemoglobin (g/L)	137 ± 22 (71-166)	146 ± 17 (104-180)	0.05
Hematocrit (L/L)	0.40 ± 0.07 (0.21-0.51)	0.46 ± 0.05 (0.36-0.54)	<0.001
Mean corpuscular volume (fL)	113 ± 10 (95-130)	95 ± 9 (75-110)	<0.001
Mean corpuscular hemoglobin (pg)	38.4 ± 4.3 (30.7-49.4)	30.48 ± 4.1 (21.2-36.4)	<0.001
Mean corpuscular hemoglobin concentration (g/L)	339 ± 16 (305-380)	319 ± 20 (271-356)	<0.001
Platelet counts (x 10^9/L)	318 ± 92 (157-511)	287 ± 64 (181-422)	0.12

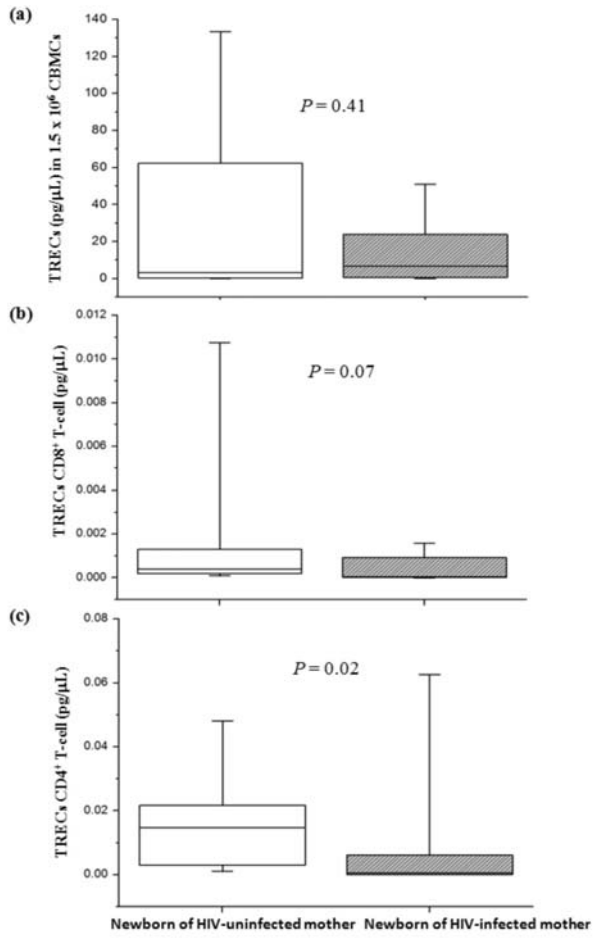

FIG. 1 *T-cell receptor excision circles (TRECs) levels of newborns of HIV-infected and uninfected mothers. (a) TRECs in CBMCs, (b) TRECs CD8⁺ T-cell, (c) TRECs CD4⁺ T-cell. TRECs levels in CBMCs were analyzed from 26 and 49 newborns of HIV-infected and uninfected mothers, respectively while TRECs CD8⁺ and CD4⁺ T-cell were analyzed from 15 and 12 newborns of HIV-infected and uninfected mothers, respectively.*

Our data are reassuring, ARV prophylaxis dose seem to significantly alter hematological indices because the mean MCV in newborns of HIV-infected mother was significantly higher than those of HIV-uninfected mothers. Moreover, some newborns (31%) of HIV-infected mother had MCV higher than the normal upper limit value (120 fL). In present study, all HIV-infected mother received ZDV and 3TC, which have been reported to induce macrocytic anemia [10, 23]. Antiretroviral drugs are routinely prescribed during the second trimester, in which hematopoiesis and lymphopoiesis are active, i.e., hepatic hematopoiesis and lymphopoiesis, spleen development, thymic education and bone marrow development. The administration of ARV drugs during the critical window of hematopoiesis and lymphopoiesis may affect the generation of these precursors [12]. Therefore, an impairement of hematopoiesis and lymphopoiesis may have contributed to the hematopoietic alteration and the reduction of thymic output, respectively. The decrease of $CD4^+$-TRECs levels observed in the present study was consistent with the previous study by Clerici, *et al*. [22] that showed $CD4^+$/ $45RA/62^+$ (naïve lymphocytes) in HIV-uninfected newborns of HIV-infected mothers received ZDV for prevention of HIV-MTCT were significantly lower than those of newborns of HIV-uninfected mothers. In contrast, Kolte, *et al*. [24] showed that thymic size but not thymic function (TRECs $CD4^+$ T-cell) in HIV-uninfected newborns of HIV-infected mothers received ARV drugs [ZDV/ plus 3TC and LPV/r or Nevirapine (NVP)] for prevention of HIV-MTCT was significantly lower than those of HIV-uninfected mothers [24]. While our cohorts were newborns, Kolte's cohorts were children with age of 15 months, that was probably when the side effect of ARV drugs resolved. Moreover, the maternal ethnicities between the two groups of children were different [24]. There are many parameters that have been shown to be associated with the hematological variables such as maternal ethnicity, drug use, maternal $CD4^+$ T-cell count at delivery, mode of delivery and also infant gestation age, birthweight and sex [25, 26]. In the current study, these factors were controlled by matching of maternal ethnicity, maternal age at delivery, gestational age, mode of delivery, fetal sex and birthweight between the test group and control group.

WBC counts, absolute neutrophil counts, absolute lymphocyte counts and platelet counts in newborns of HIV-infected mothers were similar to those of HIV-uninfected mothers (***Table* II**). These results were consistent with the previous study by Bunders, *et al*. [27] that showed the levels of WBC counts, absolute neutrophil counts, absolute lymphocyte counts and platelet counts measured at birth in HIV-1/ARV-exposed infants were not different from those in matched comparison group. However, a lower WBC counts, absolute neutrophil counts in HIV-1/ARV-exposed infants were observed at 5 weeks of age while a lower level of hemoglobin in these infants were observed at birth and 5 weeks of age. Thus, further studies are needed to evaluate how long the hematological alteration and impaired thymic function persist.

The present study has a limitation in the limited volume of cord blood collected, thus levels of TRECs in $CD4^+$ and $CD8^+$ T-cells could be analyzed in only 12 and 15 samples of newborns of HIV-uninfected and infected mothers, respectively. Moreover, it was impossible to

analyze the levels of TRECs in memory or naïve $CD4^+$ and $CD8^+$ T-cell sub-populations ($CD45RO^+$ and $CD45RA^+$), which are the immune resources. Although, the hemoglobin and hematocrit in newborns of HIV-infected mothers were significantly lower than those of HIV-uninfected mothers. We also found that, mean levels of these two hematological parameters in both groups were lower than normal range levels. These lower levels might have caused from the hematologic genetic disorders such as thalassemia and G-6-PD deficiency, frequently found in Thai population [28]. However, the hematologic genetic disorders were not used as a variable factor in our study.

In summary, our study indicates that ARV drugs (ZDV plus 3TC and LPV/r) for prevention of HIV-MTCH altered the hematological parameters and impaired thymic function (TRECs $CD4^+$ T-cell) in newborns of HIV-infected mothers. These phenomena may impact the quality of life including growth, development, vaccination responses and susceptibility to infections of infants. Therefore the long-term effects of these drugs in larger population are needed to be clarified.

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Contributors: RW and NP: patient enrolment, data acquisition, data analysis, laboratory analysis and drafting of manuscript; PS and DK: data analysis and interpretation and critical revision of the manuscript; PO, PSV and SP: concept and design, data acquisition, data analysis and interpretation and critical revision of the manuscript. All the authors were involved in preparation of the manuscript.
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REFERENCES

1. Quinn TC, Overbaugh J. HIV/AIDS in women: an expanding epidemic. Science. 2005;308:1582-3.
2. Burns DN, Mofenson LM. Paediatric HIV-1 infection. Lancet. 1999;354:SII1-6.
3. European Collaborative Study. Mother-to-child transmission of HIV infection in the era of highly active antiretroviral therapy. Clin Infect Dis. 2005;40:458-65.
4. Cooper ER, Charurat M, Mofenson L, Hanson IC, Pitt J, Diaz C, *et al.* Combination antiretroviral strategies for the treatment of pregnant HIV-1-infected women and prevention of perinatal HIV-1 transmission. J Acquir Immune Defic Syndr. 2002;29:484-94.
5. Baroncelli S, Pinnetti C, Genovese O, Tamburrini E, Floridia M. Hematological effects of zidovudine prophylaxis in newborn infants with and without prenatal exposure to zidovudine. J Med Virol. 2011;83:551-6.
6. El Beitune P, Duarte G. Antiretroviral agents during pregnancy: consequences on hematologic parameters in HIV-exposed, uninfected newborn infant. Eur J Obstet Gynecol Reprod Biol. 2006;128:59-63.
7. Feiterna-Sperling C, Weizsaecker K, Buhrer C, Casteleyn S, Loui A, Schmitz T, *et al*. Hematologic effects of maternal antiretroviral therapy and transmission prophylaxis in HIV-1-exposed uninfected newborn infants. J Acquir Immune Defic Syndr. 2007;45:43-51.
8. Gribaldo L, Malerba I, Collotta A, Casati S, Pessina A. Inhibition of CFU-E/BFU-E by 3'-azido-3'-deoxythymidine, chlorpropamide, and protoporphirin IX zinc (II): a comparison between direct exposure of progenitor cells and long-term exposure of bone marrow cultures. Toxicol Sci. 2000;58:96-101.
9. Pinnetti C, Baroncelli S, Molinari A, Nardini G, Genovese O, Ricerca BM, *et al.* Common occurrence of anaemia at the end of pregnancy following exposure to zidovudine-free regimens. J Infect. 2011;63:144-50.
10. Moyle G, Sawyer W, Law M, Amin J, Hill A. Changes in hematologic parameters and efficacy of thymidine analogue-based, highly active antiretroviral therapy: a meta-analysis of six prospective, randomized, comparative studies. Clin Ther. 2004;26:92-7.
11. Le Chenadec J, Mayaux MJ, Guihenneuc-Jouyaux C, Blanche S. Perinatal antiretroviral treatment and hematopoiesis in HIV-uninfected infants. AIDS. 2003;17:2053-61.
12. Pacheco SE, McIntosh K, Lu M, Mofenson LM, Diaz C, Foca M, *et al.* Effect of perinatal antiretroviral drug exposure on hematologic values in HIV-uninfected children: An analysis of the women and infants transmission study. J Infect Dis. 2006;194:1089-97.
13. Watson WJ, Stevens TP, Weinberg GA. Profound anemia in a newborn infant of a mother receiving antiretroviral therapy. Pediatr Infect Dis J. 1998;17:435-6.
14. Bains I, Thiebaut R, Yates AJ, Callard R. Quantifying thymic export: combining models of naive T cell proliferation and TCR excision circle dynamics gives an explicit measure of thymic output. J Immunol. 2009;183:4329-36.
15. Steinmann GG, Klaus B, Muller-Hermelink HK. The involution of the ageing human thymic epithelium is independent of puberty. A morphometric study. Scand J Immunol. 1985;22:563-75.
16. Livak F, Schatz DG. T-cell receptor alpha locus V(D)J recombination by-products are abundant in thymocytes and mature T cells. Mol Cell Biol. 1996;16:609-18.
17. Clark DR, Repping S, Pakker NG, Prins JM, Notermans DW, Wit FW, *et al.* T-cell progenitor function during progressive human immunodeficiency virus-1 infection and after antiretroviral therapy. Blood. 2000;96:242-9.
18. Dam Nielsen S, Kjaer Ersboll A, Mathiesen L, Nielsen JO, Hansen JE. Highly active antiretroviral therapy normalizes the function of progenitor cells in human immunodeficiency virus-infected patients. J Infect Dis. 1998;178:1299-305.
19. Nielsen SD, Jeppesen DL, Kolte L, Clark DR, Sorensen TU, Dreves AM, *et al.* Impaired progenitor cell function in HIV-negative infants of HIV-positive mothers results in decreased thymic output and low CD4 counts. Blood. 2001;98:398-404.
20. Winichagoon P. Prevention and control of anemia: Thailand

experiences. J Nutr. 2002;132:862-6.
21. Ometto L, De Forni D, Patiri F, Trouplin V, Mammano F, Giacomet V, *et al.* Immune reconstitution in HIV-1-infected children on antiretroviral therapy: role of thymic output and viral fitness. AIDS. 2002;16:839-49.
22. Clerici M, Saresella M, Colombo F, Fossati S, Sala N, Bricalli D, *et al.* T-lymphocyte maturation abnormalities in uninfected newborns and children with vertical exposure to HIV. Blood. 2000;96:3866-71.
23. Eyer-Silva WA, Arabe J, Pinto JF, Morais-De-Sa CA. Macrocytosis in patients on stavudine. Scand J Infect Dis 2001;33:239-40.
24. Kolte L, Ryder LP, Albrecht-Beste E, Jensen FK, Nielsen SD. HIV-infected patients with a large thymus maintain higher CD4 counts in a 5-year follow-up study of patients treated with highly active antiretroviral therapy. Scand J Immunol. 2009;70:608-13.
25. Bunders M, Thorne C, Newell ML. Maternal and infant factors and lymphocyte, CD4 and CD8 cell counts in uninfected children of HIV-1-infected mothers. AIDS. 2005;19:1071-9.
26. Rodriguez EM, Mofenson LM, Chang BH, Rich KC, Fowler MG, Smeriglio V, *et al.* Association of maternal drug use during pregnancy with maternal HIV culture positivity and perinatal HIV transmission. AIDS. 1996;10:273-82.
27. Bunders MJ, Bekker V, Scherpbier HJ, Boer K, Godfried M, Kuijpers TW. Haematological parameters of HIV-1-uninfected infants born to HIV-1-infected mothers. Acta Paediatr. 2005;94:1571-7.
28. Tanphaichitr VS, Mahasandana C, Suvatte V, Yodthong S, Pung-amritt P, Seeloem J. Prevalence of hemoglobin E, alpha-thalassemia and glucose-6-phosphate dehydrogenase deficiency in 1,000 cord bloods studied in Bangkok. Southeast Asian J Trop Med Public Health. 1995;26:271-4.