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S. Tanawattanacharoen

P. Suchartchan

S. Manotaya

B. Uerpairojkit

Y. Tanirandorn

*See next page for additional authors*

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## Hematocrit vs. complete blood count with red bloodcell indices as a hematologic screening for anemia in pregnant women: Which one is more proper?

### Authors

S. Tanawattanacharoen, P. Suchartchan, S. Manotaya, B. Uerpairojkit, Y. Tanirandorn, and D. Charoenvidhya

## นิพนธ์ต้นฉบับ

# Hematocrit vs. complete blood count with red blood cell indices as a hematologic screening for anemia in pregnant women: Which one is more proper ?

Somchai Tanawattanacharoen\* Pranee Sucharitchan\*\*

Saknan Manotaya\* Boonchai Uerpairojkit\*

Yuen Tannirandom\* Dhiraphongs Charoenvidhya\*

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**Objective** : *To determine proper and useful hematologic screening for anemia in pregnant women.*

**Methods** : *A prospective longitudinal study of 217 pregnant women recruited from the antenatal care clinic at King Chulalongkorn Memorial Hospital. Gestational age was determined by normal last menstrual period, and confirmed by crown-rump length through transvaginal ultrasound in every case. Blood samples were taken 1 week after first visit for complete blood count (CBC) with red blood cell (RBC) indices, hemoglobin typing and serum ferritin. The detection of anemia by CBC with RBC indices was compared with routine hematocrit (Hct) screening.*

**Results** : *Of the total number of 217 pregnant women, only one case was diagnosed to have anemia by using routine hematocrit examination, while there were 10 cases of anemic patients based on automated*

\* Department of Obstetrics and Gynaecology, Faculty of Medicine, Chulalongkorn University

\*\*Department of Medicine, Faculty of Medicine, Chulalongkorn University

*Hb < 10 g/dl or Hct < 30 %. Among the remaining 207 pregnant women, there were 71 cases with mean corpuscular volume (MCV) < 80 fl or mean corpuscular hemoglobin (MCH) < 27 pg. In this group, we found 14 iron deficient women, 5 cases of  $\beta$ -thalassemic trait 35 cases of hemoglobin (Hb) E-trait and 6 cases of homozygous Hb E. Moreover, in the group of patients with normal RBC indices, there were 35 cases of iron deficiency, 3 cases of  $\beta$ -thalassemic trait, and 9 cases of Hb E-trait.*

**Conclusion** : *CBC with RBC indices should be documented as a proper and useful screening for anemia in pregnant women. This may also be a useful aid in the prenatal diagnosis of fetal hemoglobinopathies.*

**Key words** : *CBC, Anemia, Pregnancy.*

Reprint request : Tanawattanacharoen S, Department of Obstetrics and Gynaecology,  
Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.

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สมชาย ธนวัฒนาเจริญ, ปราณีย์ สุจริตจันทร์, ศักนัน มะโนทัย, บุญชัย เอื้อไพโรจน์กิจ, เยือน  
ตันนิรันดร, ธีระพงศ์ เจริญวิทย์. ความเข้มเลือดกับการตรวจนับเม็ดเลือดและหาค่าดัชนีเม็ด  
เลือดแดงในการตรวจกรองภาวะโลหิตจางในสตรีตั้งครรภ์: วิธีใดเหมาะสมกว่า ? จุฬาลงกรณ์  
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- วัตถุประสงค์** : เพื่อศึกษาหาวิธีการตรวจกรองภาวะโลหิตจางในสตรีตั้งครรภ์ที่เหมาะสม  
และได้ประโยชน์สูงสุด
- วิธีการศึกษา** : การศึกษาแบบไปข้างหน้า ในสตรีตั้งครรภ์จำนวน 217 รายที่มาใช้บริการ  
ตรวจฝากครรภ์ที่โรงพยาบาลจุฬาลงกรณ์ โดยแต่ละรายได้รับการตรวจ  
ยืนยันอายุครรภ์โดยใช้การคำนวณจากวันที่มีระดูปกติครั้งสุดท้าย ร่วมกับ  
การตรวจ *Crown-rump length* หลังจากการฝากครรภ์ครั้งแรก 1 สัปดาห์  
สตรีทุกรายได้รับการเจาะเลือดเพื่อตรวจ *CBC with RBC indices*,  
*Hemoglobin typing* และค่า *Serum ferritin* เปรียบเทียบประสิทธิภาพใน  
การตรวจกรองภาวะโลหิตจางแบบต่าง ๆ โดยการตรวจ *CBC with RBC*  
*indices* กับ *Hct* ซึ่งใช้อยู่ตามปกติ
- ผลการศึกษา** : การตรวจ *Hct* ที่ใช้อยู่ตามปกติสามารถวินิจฉัยภาวะโลหิตจางในสตรีกลุ่ม  
ที่ทำการศึกษาได้เพียง 1 ราย ในขณะที่เมื่อใช้เกณฑ์ *Automated HB* <  
10 g/dl หรือ *Hct* < 30% สามารถวินิจฉัยภาวะโลหิตจางได้เพิ่มเป็น 10  
ราย ในสตรีตั้งครรภ์อีก 207 ราย ที่เหลือตรวจพบว่ามีเม็ดเลือดแดงซึ่งมี  
ขนาดเล็ก (*MCV* < 80 fl) หรือ ความเข้มข้นน้อย (*MCH* < 27 pg) ถึง  
71 ราย โดยในกลุ่มนี้ได้รับการตรวจพบว่าเป็นภาวะขาดธาตุเหล็ก 14 ราย  
เป็น  $\beta$ -thalassemic trait 5 ราย เป็น *Hb E-trait* 35 ราย และเป็น  
homozygous *Hb E* 6 ราย ในขณะที่สตรีตั้งครรภ์กลุ่มที่มีเม็ดเลือดแดง  
ขนาดและความเข้มข้นปกติ ได้รับการตรวจพบว่าเป็นภาวะขาดธาตุเหล็ก  
35 ราย เป็น  $\beta$ -thalassemic trait 3 ราย และเป็น *Hb E-trait* 9 ราย
- สรุป** : การตรวจ *CBC with RBC indices* น่าจะเป็นวิธีการตรวจกรองหาโลหิต  
จางในสตรีตั้งครรภ์ที่เหมาะสมและได้ประโยชน์ และอาจเป็นเครื่องมือ  
สำคัญที่จะนำไปสู่การตรวจวินิจฉัยก่อนคลอด เพื่อหาความผิดปกติของ  
*Hemoglobin* ของทารกต่อไป

Pregnancy induces physiological changes that often confuse the diagnosis of several hematological disorders and the assessment of their treatment. This is especially true for anemia. One of the most significant changes is that of blood volume expansion by a mean of 50 percent. Plasma volume increases disproportionately compared with red blood cell mass, resulting in physiological decrease in hematocrit.<sup>(1,2)</sup> The disproportion between the rates at which plasma and erythrocytes are added to the maternal circulation is normally greatest during the second trimester.

Any disorders causing anemia encountered in childbearing-age women may complicate pregnancy. The most common cause of anemia during pregnancy is iron deficiency.<sup>(3)</sup> Another common cause in Thailand is hemoglobinopathy, especially thalassemia.

Thalassemia is one of the major public health problems in Thailand. The two most common type are  $\alpha$ - and  $\beta$ -thalassemia, both of which affect the synthesis of Hb A. Hb E and Hb CS are also prevalent.<sup>(4)</sup> The frequencies are 5-20 percent for  $\alpha$ -thalassemia, 1-9 percent for  $\beta$ -thalassemia, 10-15 percent for Hb E and at least 4 percent for Hb CS. The interaction between these abnormal genes results in over 60 thalassemia syndromes.<sup>(5,6)</sup>

Efforts in carrier detection, genetic counseling and prenatal diagnosis should be encouraged. One effective way to screen the population for carriers can be done in pregnant women by measurement of mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) by standard electronic cell counter or RBC indices.

This study aimed to determine proper and useful hematologic screening for anemia in pregnant women. Hematocrit, a standard screening method at King Chulalongkorn Memorial Hospital, were compared with CBC with RBC indices.

### Materials and Methods

Two hundred and seventeen pregnant women were recruited into the study from our antenatal clinic. Inclusion criteria were (1) regular menstrual cycle with certain last menstrual period, (2) gestational age less than 12 completed weeks, (3) informed consent was provided. Gestational age was determined by last normal menstrual period and confirmed by crown-rump length through transvaginal ultrasound in every case. Exclusion criteria were (1) women with a date-size discrepancy of more than 7 days, (2) multifetal pregnancy, or (3) non-viable pregnancy. Hematocrit, as well as other serologic tests, was routinely tested at the initial visit. A blood sample was taken 1 week later for CBC with RBC indices, Hb electrophoresis and serum ferritin. Two hundred mg of ferrous fumarate daily was prescribed in each case. Pregnant women with abnormal Hb typing were informed and counseled. Partner evaluation was suggested. All women were followed until delivery.

Microcentrifugation machine was used to determine hematocrit values. CBC with RBC indices were determined according to the standard laboratory procedures with the use of Coulter<sup>R</sup> (MAXM, Miami, Florida, USA). Serum ferritin were measured with the Microplate Ferritin kit (Bio-Rad laboratories, USA). Anemia was defined as hematocrit less than 30 %

and/or hemoglobin concentration less than 10 grams per dL. Iron deficiency was diagnosed when serum ferritin levels were less than 20 ng/dl. Iso-electric focusing electrophoresis was used to analyze Hb electrophoresis by the Resolve system (EG&G Wallac, Akron, Ohio, USA).

### Results

The mean age of the studied population was  $27.4 \pm 5.1$  years (range 15 - 40). The mean gestational age was  $10.5 \pm 1.5$  weeks (range 7 - 12). Routine Hct obtained at first visit was  $36.8 \pm 2.2$  % (range 26 - 44). There was only one pregnant woman who had anemia from routine Hct screening (Hct = 26%) while there were 10 cases of anemic

patients based on automated Hb < 10 g/dL or Hct < 30 %. The results of CBC with RBC indices in both anemic and non-anemic groups is shown in Table 1.

Serum ferritin was obtained only in 214 cases (one in the anemic group and 2 the in the non-anemic group were missing). Mean serum ferritin in the anemic and non-anemic groups were  $60.6 \pm 47.8$  (range 15 - 135) and  $50.6 \pm 57.2$  (range 4 - 441) ng/dL, respectively.

Detailed results information for ten anemic pregnant women is shown in Table 2.

Among the remaining 207 pregnant women, there were 71 cases who had MCV < 80 fl or MCH < 27 pg. Hematologic disorders of both groups are classified in Table 3.

**Table 1.** CBC with RBC indices of anemic and non-anemic pregnant women.

	Anemic (N = 10) Mean $\pm$ S.D. (range)	Non-anemic (N = 207) Mean $\pm$ S.D. (range)
Hb (g/dL)	$9.7 \pm 0.6$ (8.5 - 10.4)	$12.0 \pm 0.9$ (10.1 - 15.6)
Hct (%)	$29.2 \pm 1.1$ (26.5 - 30.6)	$35.9 \pm 2.7$ (30.0 - 46.1)
RBC count	$4.1 \pm 0.5$ (3.6 - 4.8)	$4.3 \pm 0.5$ (3.2 - 5.7)
MCV (fL)	$71.9 \pm 8.8$ (59.1 - 81.4)	$84.0 \pm 7.4$ (64.9 - 98.8)
MCH (pg)	$23.9 \pm 3.5$ (18.9 - 27.6)	$28.2 \pm 3.0$ (20.4 - 37.4)
MCHC (g/dL)	$33.1 \pm 1.2$ (30.9 - 34.8)	$33.4 \pm 0.9$ (31.1 - 36.3)
RDW (%)	$15.9 \pm 5.8$ (12.6 - 31.8)	$12.9 \pm 1.1$ (11.0 - 18.6)

Table 2. Information of anemic pregnant women.

No.	Hct at ANC (%)	Hct (%)	Hb (g/dL)	Hemoglobin typing	Serum ferritin
1	38	26.5	8.5	A <sub>2</sub> A	120
2	36	28.4	9.9	A <sub>2</sub> A	135
3	39	28.8	9.4	E-trait	62
4	36	28.9	9.9	E-trait	48
5	36	29.3	9.0	Homozygous E	105
6	36	29.5	10.0	E-trait	15
7	40	29.8	10.0	β-trait	17
8	37	29.8	10.4	E-trait	25
9	36	30.0	9.9	Homozygous E	18
10	36	30.6	9.9	A <sub>2</sub> A	-

Table 3. Hematologic disorders of abnormal and normal RBC indices pregnant women.

	MCV < 80 or MCH < 27 (N = 71)*	MCV ≥ 80 & MCH ≥ 27 (N = 136)**
Iron deficiency	14	35
Abnormal Hb typing	46	12
β-thalassemic trait	5	3
Hb E-trait	35	9
Homozygous Hb E	6	-

\* 7 cases had both Iron deficiency and abnormal Hb typing

\*\* 2 cases had both Iron deficiency and abnormal Hb typing

## Discussion

There are several important reasons for screening a prenatal population at risk for inherited hemoglobinopathy. First, by establishing a diagnosis, one may avoid inappropriate treatment. Second, in the patients whose disorder places her at increased

obstetric risk, identifying the high-risk pregnancy will allow appropriate management. Third, when the potential for a severely affected offspring exists, appropriate partner screening and referral for genetic counseling should be initiated.



In this study, only one case was diagnosed to have anemia (Hct = 26%) by our routine Hct screening at the antenatal care clinic. Even when screened based on automated Hb < 10 g/dl or Hct < 30 %, many cases with abnormal Hb typing and iron deficiency were missed. From the non-anemic group (207 cases), 71 cases were found who had MCV < 80 fl or MCH < 27 pg. Among this group, 14 cases of iron deficiency and 46 cases of abnormal Hb were diagnosed. However, 35 cases of iron deficiency and 12 cases of abnormal Hb typing were missed in those who had normal values of RBC indices.

The reason that not all cases of iron deficiency could be detected may be because there are three stages of iron deficiency. The first is a reduction of iron stores and only decreased serum ferritin is shown. The second stage starts when iron stores are depleted but anemia has not yet resulted. The third and most severe stage is manifested as overt microcytic anemia which reflects by a low hemoglobin level, a low serum ferritin, and a decrease in RBC indices. And the first and second stage of iron deficiency are seen commonly in the first trimester of pregnancy.<sup>(7)</sup> In our study a total of 49 cases (22.6%) were diagnosed as iron deficient. In this group, 3 cases showed overt anemia, 11 cases had abnormal RBC indices without anemia, reduced serum ferritin concentrations were the only finding in 35 cases.

A number of pregnant women who had MCV < 80 fl or MCH < 27 pg had normal Hb typing and normal iron stores might have some abnormalities of  $\alpha$ -globin production.<sup>(8)</sup> Diagnosis of  $\alpha$ -thalassemic traits couldn't be conclusively determined in this study because those cases weren't confirmed by polymerase chain reaction (PCR) technique. However,

when CBC with RBC indices was used as a hematologic screening for anemia, a high risk group of such conditions can be detected by the appropriate technology and prenatal diagnosis will then be considered.

Although not all anemic disorders in pregnant women could be diagnosed by CBC with RBC indices screening, the majority of them were detected. Therefore, CBC with RBC indices should be considered a proper and useful screening for anemia in pregnant women. This may also be a useful aid in the prenatal diagnosis of fetal hemoglobinopathies in the future. Nevertheless, if prenatal diagnosis is to be performed, four prior steps are necessary. First, the carrier must be identified through screening. Second, the partner must be tested. Third, if the partner is positive, the couples must understand their risks and options. And last, the couple must choose prenatal diagnosis on voluntary basis.<sup>(9)</sup>

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