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Blood Smear Interpretation for Screening of Hemoglobin Disorders in Thai Pregnant Women, is it Effective ?

การคัดกรองความผิดปกติของฮีโมโกลบินในหญิงตั้งครรภ์โดย Blood Smear

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Any hematological disorders encountered in women of child-bearing age may complicate the pregnancy. Apart from iron deficiency anemia, one common anemia in pregnancies in Thailand is due to hemoglobin disorders. The two most common types are hemoglobin E and beta thalassemia, which affect the synthesis of hemoglobin. These genetic abnormalities cause many systemic effects. Chronic hemolytic anemia results from aberrant fragile red blood cells. Limitations of normal daily activity, jaundice, hepatosplenomegaly, osteoporosis and facial abnormalities are common.

At present, 500,000 Thai people suffer from these diseases-of these 12,000 belong to the neonatal group, which is approximately 1.2% of the newborns in each year. Therefore, thalassemia and hemoglobinopathy are major health problems in Thailand.[1] This problem affects not only public health but also the economy of the country. If no method for control was implemented the number of patients and carriers will increase and social problem will be the result. Carrier detection, genetic counseling and prenatal diagnosis should be encouraged.[2]

At present, apart from routine hematocrit detection as part of the antenatal program, another interesting method for screening the population for carriers especially among pregnant women is blood smear interpretation. This study has been performed to determine the proper and useful hematological screening for hemoglobin disorders in pregnant women by blood smear interpretation.

Materials and methods

One hundred and fourteen pregnant women who underwent routine antenatal laboratory screening check-up from the Out-Patient Division, King Chulalongkorn Memorial Hospital were included in this study. Inclusion criteria were 1) regular

menstrual cycle with certain last menstrual period, 2) confirmed pregnancy and 3) informed consent was provided. Exclusion criteria were 1) non-viable pregnancy, 2) multifetal pregnancy and 3) informed consent was not provided.

Hematolocrit (Hct), as well as other serological tests, were routinely performed at the antenatal clinic. Our investigations were then performed. A venous blood specimen from each subject was collected in EDTA tubes and analyzed according to the following categories. Blood smear was performed for each sample and then interpretation was done by agreement of 2 in 3 experienced physicians.

Hemoglobin typing by cellulose acetate electrophoresis (Helena Laboratory : Beaumont, Texas) served as a confirmation test. Comparison with control hemoglobin electrophoresis was performed in each specimen. Repeated confirmation based on manual blood smear interpretation by a team of experienced medical technologists was also performed. The results of each category were collected and analyzed. Diagnostic activity was determined as sensitivity, specificity, false positive and false negative values of the tests. Descriptive statistical analysis was used when appropriate.

Result

Based on the automated hematology analyzer, blood indices obtained from the automated hematology analyzer all total subjects are presented in Table 1. Based on confirmation tests there were 36 cases (31.6%) with hemoglobin disorders as presented in Table 2. The most common abnormality was the hemoglobin E trait (EA) disorder.

Of all 114 subjects, only 16 cases were diagnosed with anemia based on automated hemoglobin or hematocrit criteria (Hb < 10 gram/deciliter or Hct < 30 %). Considering of these 16 subjects, only 14 cases presented abnormal blood picture suspected for hemoglobin disorders. Of this group, only 5 cases showed hemoglobin disorders and all were diagnosed as hemoglobin E trait in 1 case and homozygous hemoglobin E in 4 cases based on the confirmation tests. The other 9 cases presented a normal hemoglobin pattern with iron deficiency disorder.

Using blood smear interpretation, 67 samples

Table 1 Blood indices from automatod analyzer

	Hemoglobin (gram/deciliter)	Hematocrit (%)	Mean corpuscular volume (femtoliter)	Red blood cell distribution width (%)	Mean copuscular hemoglobin (picogram)
Mean	11.37	33.84	83.38	13.70	28.40
Standard deviation	1.14	3.34	8.91	1.22	3.40
Maximum	13.80	41.80	99.20	18.10	33.90
Minimum	8.80	26.70	60.60	11.80	13.90

Table 2 Hemoglobin patterns obtained from confirmation tests.

Patterns*	Number	Valid percentage (%)
Normal	78	68.4
Hemoglobin E trait	26	22.8
Homozygous Hemoglobin E	10	8.8

Table 3 Results of bood smear interpretation as a screening test for abnormal hemoglobin inpregnant women.

Blood smear Interpretation	Hemoglobin disordes	
	Yes	no
Suspected	22	45
Not	14	33

were suspected for hemoglobin disorders. Sensitivity, specificity, false positive and false negative of the test were 61.1%, 42.3%, 39.5% and 12.3%, respectively (Table 3).

Discussion

Based on this study, the mean hemoglobin per subject was 10.99 gram/deciliter. Although this hemoglobin level was not considered to indicate anemia in pregnancy, based on hemoglobin electrophoresis, 31.6% of all subjects had hemoglobin disorders. Hemoglobin E disorder has been the most prevalent group in our study, confirming finding that hemoglobin E disorder is the most common inherited hemoglobin disorder in Thailand. Furthermore, the prevalence in this study can match the previous data where the incidence is about 13-52%.[1, 3-5]

In this study, a screening method using blood smear interpretation was evaluated. Using this screening, sensitivity and specificity are fair. Furthermore, false positive and false negative results are frequent. But compared to hematocrit determination alone, better screening is obtained. Using only hematocrit determination, a higher number of cases are overlooked.

As for the cost-effectiveness of red blood cell index determination as a screening method for hemoglobin disorders, better results than those obtained by hemoglobin electrophoresis are found.

Although this method can provide faster results compared to other screening methods, such as the combination of osmotic fragility and Dichlorophenol Indolphenol (DCIP) test, some limitations can be observed. Firstly, this method requires the experience physician to interpret the blood smear use, which is not available in some areas such as in rural community hospitals. Secondly, it still provides

rather low sensitivity.

The standard method used in this study has been hemoglobin electrophoresis instead of column chromatography. Therefore, it is impossible to determine the valid percentage of the A_2 component in case of genotype A_2A. The decision if a case with genotype A_2A is normal or not was facilitated by comparison with the standard normal control and a repeated check by blood smear examination before judgement. Therefore, further hospital studies to determine the actual incidence of inherited hemoglobin diseases by a definite diagnostic method as DNA identification are recommended.

This study has been a prospective study, therefore, the case selection bias can be controlled. Based on this study, the addition of routine blood smear interpretation to the routine antenatal check up is recommended.

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