Diagnostic Value of Combined Parameters for α-Thalassemia-1 Screening in Pregnant Women

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Received 28 April 2004; accepted 4 August 2004

Abstract

Hb Bart's hydrops fetalis, the most severe case α-thalassemia, can be prevented with an early effective diagnosis of α-thalassemia-1 carrier pregnant women. Current commonly used diagnostic parameter is MCV or OFT. However both tests are not specific enough. Therefore, more samples are required further tests. This study aimed to investigate a parameter or combined parameters that provide(s) higher specificity and sensitivity in differential diagnosis between carriers and non-carriers of α-thalassemia-1. Blood samples were collected from 1,000 pregnant women attending an antenatal clinic, Buddhachinaraj Hospital, Phitsanulok. The EDTA anticoagulated blood was subjected to determination of RBC count, RBC indices (Hb, Hct, MCH, MCHC, MCV and RDW) by an automated blood cell analyzer, OFT and DNA extraction for PCR-based assay. Data were analyzed by ROC curves and discriminant analysis and then compared with Student's t-test. This study showed that combined parameters revealed more effective diagnosis of α-thalassemia-1 than a single parameter. Considering 100% sensitivity, the most effective combination indicator was MCH/OFT/RDW (92.4% specificity), followed by MCH/OFT/MCV (91.9% specificity), MCH/OFT (91.7% specificity), MCH/RDW/MCV (90.0% specificity), MCH/RDW (89.6% specificity) and MCH/MCV (88.5% specificity). MCH, MCV and OFT used as a single parameter exhibited % specificity of 87.3, 82.4 and 81.7%, respectively. The cut-off points of these parameters were as followed: MCH (≤ 25 pg), MCV (≤ 75 fL), RDW (≥ 14.5%) and OFT (≤ 55%). The results suggested that the parameter combinations provide better predictive values of a positive test for α-thalassemia-1 carriers with no false negative. The best combined parameter was MCH/OFT/RDW.

Keywords: α-thalassemia-1 screening, combined parameters, specificity

Introduction

The α-thalassemia is one of the most common genetic disorders that results from reduced synthesis of α-globin chains. Normal human α-globin gene cluster is located on the short arm of chromosome 16p13.3. The most common causes of α-thalassemia are deletions of one (-α) or two (--) functional α-globin genes. Alpha-thalassemia-1 (--/αα) and α-thalassemia-2 (-α/αα) are carriers with a phenotype of microcytic red blood cell (RBC). Therefore, if both partners are α-thalassemia-1 carriers, they could be at a reproductive risk of Hb

Bart's hydrops fetalis syndrome (--/--). Hb Bart's hydrops fetalis is the most severe thalassemic case and an important health problem in Southeast Asia (Greenberg, 2001). All of the fetuses with the severe type of thalassemia die either in utero or soon after birth. Approximately 75% of mothers carrying Hb Bart's hydrops fetalis develop toxemia of pregnancy (Weatherall, 1998). Hence, improved screening methods for α-thalassemia-1 in pregnant women would ultimately facilitate identification of pregnancies at risk of hydrops fetalis and prevention through a prenatal diagnosis.

Currently differentiation between α-thalassemia-1 and non-α-thalassemia-1 is made by using RBC indices; mean corpuscular volume (MCV) or mean corpuscular hemoglobin (MCH) (Clarke, 2000) or simple erythrocyte osmotic fragility test (OFT) (Sanguansermsri, 1999) as a key screening indicator. However, these parameters are not specific enough, i.e., some false negative can occur. Therefore, this study aimed to investigate more diagnostic accuracy of combined parameters in considering of sensitivity and specificity for screening of α-thalassemia-1 carriers.

Materials and Methods

Samples

This research was approved by the Human Ethical Committee, Naresuan University, Phitsanulok, Thailand. Three milliliters of blood were collected from each pregnant women (n=1,000, age $26^{+}7.4$ years) and put into an EDTA anticoagulant tube. These women were first attending an antenatal clinic at Buddhachinaraj Hospital in Phitsanulok. An aliquot of blood was used for measurement of RBC count, RBC indices (Hb, Hct, MCV, MCH, MCHC, RDW) by Sysmex KX-21 automated blood cell analyzer (Sysmex Corporation, Kobe, Japan). Ten microliters of the anticoagulated whole blood were immediately performed OFT and percentages of hemolysis were observed within 2 minutes (Sanguansermsri, 1999).

DNA Analysis

DNA was extracted from peripheral-blood leukocytes by chelex (Walsh, 1991). The α-thalassemia-1 (SEA-type) was determined by polymerase chain reaction (PCR) based method using 3 specific primers (Sanguansermsri, 1999): one forward primer (5'-GCGATCTGGGCTCTGTGTTCT-3') and two reverse primers, for non-α-thalassemia-1 (5'-GTTCCCTGAGCCCCGACACG-3') and for α-thalassemia-1 (5'-GCCTTGAACTCCTGGACTTAA-3'). Reactions were performed at 40 cycles with 94^{O}C for 30 second, 58 ^{O}C for 1 minute and 72 ^{O}C for 1 minute. The PCR products were electrophoresed in 2% agarose gel, stained with ethidium bromide and visualized with an UV transiluminator. The expected product sizes for non- α-thalassemia-1 and α-thalassemia-1 were 314 bp and 188 bp, respectively.

Data Analysis

The diagnostic value of each parameter (RBC count, Hb, Hct, MCV, MCH, MCHC, RDW, OFT) was investigated by comparing the histograms of the two cohorts of α-thalassemia-1 carriers and non-carriers. The two mean values were compared with Student's t-test. The histograms were transformed into a receiver operating characteristic (ROC) curve. This ROC curve is a plot between sensitivity (y-axis) versus false positive (x-axis), obtained for different cut-off points. Area under the curves (AUC) of the ROC curve and their 95% confidence interval (CI) were evaluated as a measure of diagnostic accuracy. A discriminant analysis was performed to identify a combination of these parameters that provides the best differentiation between α-thalassemia-1 and non-α-thalassemia-1 subjects. A stepwise selection of parameters was used to obtain the best subset of indices in the discriminant analysis.

Results

Descriptive statistics of RBC count, RBC indices and OFT for the cohort of α-thalassemia-1 carriers (n=53) and the cohort of non-α-thalassemia-1 carriers (n=947) are shown in Table 1. These parameters were significantly different between α and non-α-thalassemia-1 using Student's t-test (p-value < 0.001) and confirmed by AUCs (> 0.5). Figure 1 shows the ROC curves of each parameter. AUC indicates the relationship between sensitivity and false positive (1-specificity) of a single test. Greater AUC indicates higher sensitivity and specificity. Regarding a single parameter, MCH (AUC=0.98) was the most effective diagnosis indicator for α-thalassemia-1, followed by MCV (AUC=0.97), OFT (AUC=0.95), RDW (AUC=0.94), RBCs (AUC=0.88), MCHC (AUC=0.86), Hb (AUC=0.79) and Hct (AUC=0.68).

Table 1 RBC count, RBC indices and OFT of pregnant women with α-thalassemia-1 and non-α-thalassemia-1 and area under ROC curve (AUC) of each parameter

Parameter	α-thalassemia-1 (n=53) Mean ± SD	non-α-thalassemia-1 (n=947) Mean ± SD	P-values*	AUC
RBC ($x10^{12}$/L)	4.90 ± 0.68	4.11± 0.50	< 0.001	0.88
Hb (g/dL)	10.1 ± 1.22	11.4 ± 1.20	< 0.001	0.79
Hct (%)	31.4 ± 3.83	33.4 ± 3.46	< 0.001	0.68
MCV (fL)	64.1 ± 5.34	81.9 ± 7.73	< 0.001	0.97
MCH (pg)	20.6 ± 2.03	28.0 ± 2.95	< 0.001	0.98
MCHC (g/dL)	32.1 ± 1.47	34.2 ± 1.64	< 0.001	0.86
RDW (%)	18.5 ± 3.72	14.4 ± 1.67	< 0.001	0.94
OFT (%)	22.3 ± 14.1	71.6 ± 20.0	< 0.001	0.95

Note: * P-values of mean comparison with Student's t-test

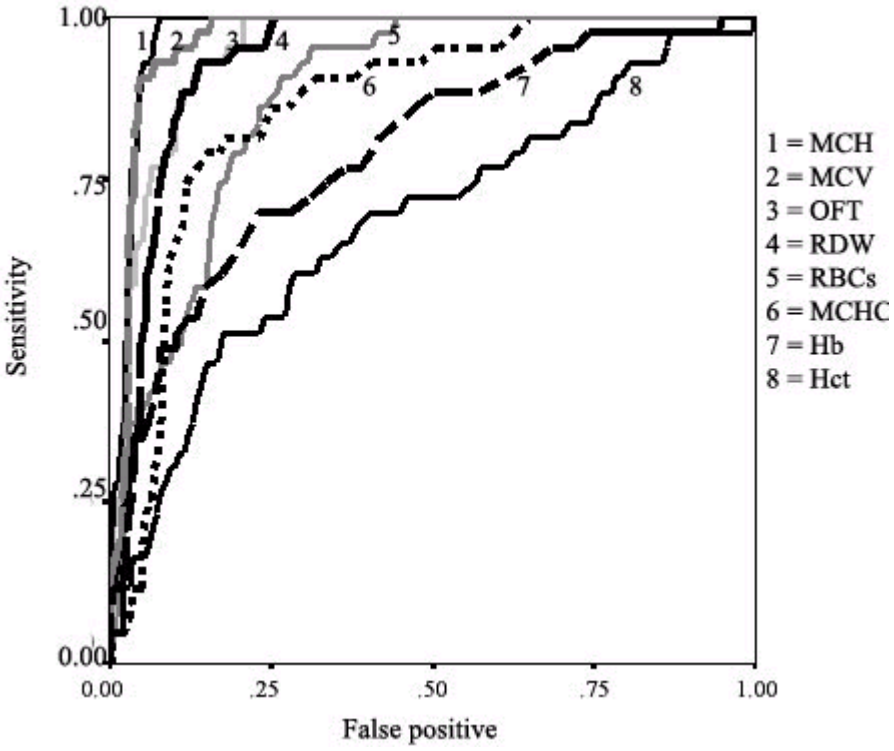

Figure 1 ROC curves of RBC count, RBC indices and OFT in diagnosis α-thalassemia-1 carriers: ROC curves are constructed from plotting sensitivity (true positive) and the false positive.

The parameters selected by the discriminant analysis were MCH, OFT and RDW. When using only RBC indices, MCH and RDW were selected. Therefore, the parameters used to look for an optimal combination were MCH, MCV, RDW, and OFT. Because of the precision of each measurement, a cut-off point of the individual parameter was selected. The cut-off points provide 100% sensitivity for diagnosis of α-thalassemia-1 (all α-thalassemia-1 carriers can be detected). The cut-off points of MCH, MCV, OFT and RDW were 25 pg, 75 fL, 55%, 14.5%, respectively. The specificity of a single and of the combined parameters is shown in Table 2. The best indicator for α-thalassemia-1 detection (100% sensitivity) was the combination of MCH/OFT/RDW (92.4% specificity). While MCH, a single parameter provided the highest specificity of only 87.3%.

Table 2 Specificity (%) of each parameter and their combination for diagnosis α-thalassemia-1

Parameter (cut-off point)	% specificity
MCH / OFT / RDW (25/55/14.5)	92.4
MCH / OFT / MCV (25/55/75)	91.9
MCH / OFT (25/55)	91.7
MCH / RDW / MCV (25/14.5/75)	90.0
MCH / RDW (25/14.5)	89.6
MCH / MCV (25/75)	88.5
MCH (25)	85.0
MCV (75)	81.5
OFT (55)	78.3
RDW (14.5)	64.0

Discussion and Conclusion

RBC count, RBC indices and OFT were measured in 1,000 pregnant women attending an antenatal clinic at Buddhachinaraj Hospital in Thailand. Their genotypes were classified into α-thalassemia-1 carriers ($--^{SEA}/\alpha\alpha$; n = 53) and non-α-thalassemia-1 carriers (normal = 577 cases, α-thalassemia-2 = 146 cases, HbCS = 53 cases, HbE = 157 cases, and β-thalassemia=14 cases; n = 947) by DNA analysis. RBC count, RBC indices and OFT were compared between groups by Student's t-test, ROC curve method and discriminant analysis.

Improving diagnosis indicators provides more accuracy prediction for screening of α-thalassemia-1 carriers and reduces the number of specific tests required. It is crucial for prenatal diagnosis and prevention of newborns with Hb Bart's hydrops fetalis. Generally, MCV of 80 fL is used to screen for thalassemia because of microcytic RBC in thalassemia patients. Actually, automated blood cell analyzers now simultaneously provide a measurement of all RBC count and RBC indices precision and accuracy. However, only data from one parameter is used in the diagnosis and the rest of the data are left unused. This study demonstrates that combined parameters provide more effective diagnosis of α-thalassemia-1 and help to decrease workload and cost of any specific tests further required in the conventional diagnosis.

We establish cut-off points for diagnosis of α-thalassemia-1 of MCH, MCV, RDW and OFT as ≤ 25 pg, ≤ 75 fL, ≥ 14.5% and ≤ 55% , respectively. The combination of MCH/OFT/RDW exhibited the best diagnostic parameter with 100% sensitivity and 92.4% specificity. In summary, we conclude that the combined parameters improve the predictive value of a positive test for α-thalassemia-1 carriers with no false negative. This will provide an effective screening, decrease workload and cost in prevention and control of severe thalassemia program.

Acknowledgements

We acknowledge Dr. Warong Dechgitvigrom and medical staffs at Buddhachinaraj Hospital for blood collection assistance; Dr. Jo Hermans for his medical statistics guidance and manuscript improvement; and Medical Technologists at Faculty of Medicines, Chiang Mai University and at Health Sciences Research Institute, Naresuan University for their technical assistance and those who contributed by any ways in this project. We particularly thank those who donated their blood for this study and the Faculty of Pharmaceutical Sciences, Naresuan University for financial support.

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