



MCV-MCH Value and One Tube Osmotic Fragility Test (OTOFT) Method for Thalassemia Trait Detection in Pregnancies

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Abstract

The purpose of this study was to evaluate the prevalence of carrier thalassemia on pregnant women population who visit to antenatal clinic Hasan Sadikin Hospital, Bandung, Indonesia. Design study was cross sectional that 219 blood samples have collected in tubes containing EDTA anticoagulant. Sample examined for routine blood analysis and OTOFT method. If OTOFT method result was positive that confirm with alkaline Hemoglobin electrophoresis. 17 (7.76%) suspected carrier thalassemia were positive OTOFT method. Samples with OTOFT method positive have average values of Hemoglobin, MCV, and MCH were 10.88 ± 1.61 g/dL, 82.64 ± 8.66 fL, and 26.42 ± 3.99 pg, that less than negative OTOFT method group. Average concentration of HbA2 Alkaline electrophoresis was 5.4%, more than common normal value could be concluded suspected carrier β thalassemia. Population of suspected carrier thalassemia was 7.76%. Combination MCV, MCH value and OTOFT method can use as simple screening tool for detection thalassemia trait in primary level.

Keywords: Thalassemia, pregnant women, OTOFT

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Introduction

Decrease of synthesis of globin chains constitute thalassemia with most types were the type α and β thalassemia. Alpha thalassemia was due to mutations in genes that regulate the production of α -globin chains, whereas β thalassemia is due to mutations of genes that form the β -globin chain [1-5]. Thalassemia inherited from both parents who each carry a thalassemia trait. If two person with trait thalassemia marriage, the fetus has Possibility 25% chance of becoming a normal child, 50% chance to be a carrier of the trait, and 25% be people with thalassemia major [6-8]. So the marriage between two people of having trait thalassemia should be avoided.

Measuring the absolute value of the erythrocytes as the value Mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were simple screen to early detection thalassemia trait. Reference value of a person is a carrier of thalassemia trait are less than 75-80 when the MCV and MCH values when less than 26-27 [8-9]. Gomber et al (1997) recommend MCV less than 70 will be more sensitive and specific for the screening [10]. Tangvarasittichai et al, (2004) to get the cut-off point MCV <75, and MCH <25, with a specificity of 81.5% and 85.0% whereas when combined between MCV and MCH spesivitasnya value reached 88.5% [11].

Another simple way was to use tube osmotic fragility method known as one tube osmotic fragility test (OTOFT) or also known as naked eye single tube red cell osmotic fragility test (NESTROFT) [12]. At this time in Indonesia OTOFT method were not widely used. The experience of countries that have been using this method such as India and Thailand, proved to have a sensitivity and specificity were quite good. Gomber et al (1997) found OTOFT sensitivity of 95.59% and specificity 84.2% [10]. Tangvarasittichai et al, (2004) found a sensitivity of 91.7% when OTOFT combined with MCV and MCH [11]. Chow et al suggest the use of saline 0.36% because of the research results sufficiently accurate to detect trait with a detection rate of 95% for beta thalassemia [13]. In addition, this examination is easy and low cost so that it can be done through primary health care. So therefore this study to compare diagnostic the number of population thalasaemia trait on the pregnant women using hemoglobin profile with OTOFT method confirm alkaline electrophoresis. This comparison

very important to seek effective and cheap method to mass screen in country with big population.

Materials and Methods

Subjects were pregnant women who control or go to the hospital to be examined pregnancy or require treatment. Venous blood samples, is as much as 2 milliliters of blood from the median cubital vein, then put into a tube containing EDTA anticoagulant. Blood with EDTA anticoagulant was used for inspection of Hb, MCV, MCH, OTOFT and electrophoresis on a positive with OTOFT. Forms of research was a diagnostic test with a cross sectional research design. sampling from consecutive admissions of patients pregnant women who come to control to the obstetrics and gynecology department of Dr. Hasan Sadikin.

Examination procedures performed OTOFT in this study:

1. 2 mL blood take from vena cubiti mediana (note:if only will do OTOFT procedure without other examination needed, resource blood can use direct take 20 ul from finger prick with blood lancet)
2. Enter the 5 ml working solution (buffered saline 0.36%) in a test tub
3. Take the blood by as much as 20 microliter pipette (the rest of the blood was used for inspection HB, MCV, MCH& HB Electrophoresis)
4. Blood included in the test tube that has been filled reagent buffered saline 0.36%.
5. The mixture of reagents and blood homogenized, and allowed to stand at room temperature for 5 minutes.
6. The reaction results are read visually: the lines visible behind the clear tube means a negative result, if the tube behind the lines blurred because covered with a mixture of reagents and blood (indicating foggy) were positive.

Blood sample with positif OTOFT results then subsequently confirmed using Sebia's Alkaline electrophoresis Hemoglobin assay, intended for the separation of normal hemoglobins and the major hemoglobin variants at an alkaline pH. Designed to give better separation between

A and F hemoglobins than traditional agarose assays. Haemoglobins A, A₂, F, S and C can be easily separated and identified utilizing the Alkaline assay for initial screening. Because of their varying electrical charges, when placed in this electric field, the hemoglobin particles will move and separate according to their specific types and form “bands.” A comparison of these bands to a normal blood sample will assist in diagnosing whether or not the person suffers from a disorder like sickle cell anemia. Results are routinely processed through a combination of visual bands interpretation and hemoglobin quantitation utilizing the HYDRASYS™ 2 Densitometer or Phoresis Imaging System software.

Results

Results study pointed 17 of the 219 samples (7, 76%) with positive OTOFT method. All Results of 219 samples are shown in Table 1, overall mean hemoglobin was 11.05 g / dL, it shows that the average hemoglobin levels of pregnant women above 11 g / dL as anemia physiological limits. Mean while the groups of anemia (39.3%) had hemoglobin levels <11 g / dL. Range of hemoglobin value from 6.7 g / dL to 14.5 g / dL. The group of anemia with positive results in OTOFT method as many as 8 samples (3,6%) while 9 samples (4,1%) OTOFT positive in non anemia group.

Table 2 shows that in the group with OTOFT method has an average lower test results on the value of Hb, MCV, MCH and MCHC, while the number of erythrocytes and platelets higher than negative group OTOFT examination. Nevertheless only MCV and MCH Showed statistically significant. At OTOFT positive Hemoglobin has an average of 10.15 g / dL showed that suspected thalassemia trait have a tendency to anemia, although this might be due to other causes including Fe deficiencies. Besides that also obtained an average value HBA₂ 5.4% in the samples with OTOFT positive that mean were above referral limit for normal level (>3.5 %).

Table 1. General Laboratory Findings in all Subjects

Characteristics	Mean	Group	N=219 (%)	OTOFT (N=219/%)	
				(+)	(-)
1. Hemoglobin (g/dL)	11.05	< 11	86(39.3)	8 (3.6)	78 (35.7)
		≥11	133(60.7)	9 (4.1)	124 (56.6)
2. MCV (fL)	87.28	≤ 75	11 (5.0)	5 (2.3)	6 (2.7)
		> 75	208 (95.0)	12 (5.5)	196 (89.5)
3.MCH (pg)	28.2	≤ 27	61 (27.9)	10 (4.6)	51 (23.3)
		> 27	158 (72.1)	7 (3.2)	151 (68.9)
4. Erythrocytes (x 10 ⁶ /mm ³)	3.92 10 ⁶	< 3 10 ⁶	2 (0.9)	0	2 (0.9)
		3 – 4 10 ⁶	128 (58.4)	6 (2.7)	122 (55.7)
		> 4 10 ⁶	89 (40.7)	11 (5.0)	78 (35.7)
5. Platelets (x 10 ⁵ /mm ³)	2.84 10 ⁵	<1.5 10 ⁵	7 (3.2)	1 (0.4)	6 (2.8)
		1.5-4.10 ⁵	195 (89.0)	13 (5.9)	182 (83.1)
		>4.10 ⁵	17 (7.8)	3 (1.4)	14 (6.4)

Table 2. Laboratory findings in the group with OTOFT method

Characteristics	OTOFT Positive (Mean)	OTOFT Negative (Mean)	P value
Haemoglobin (g/dL)	10.8 ± 1.6	11.0 ± 1.2	> 0.05
MCV (fL)	82.6 ± 8.6	87.7 ± 5.9	< 0.05
MCH (pg)	26.4 ± 3.9	28.3 ± 2.4	< 0.05
MCHC	31.8 ± 2.1	32.4 ± 1.2	> 0.05
Erythrocytes (x10 ⁶ /mm ³)	4.1 ± 0.5	3.9 ± 0.44	> 0.05
Platelets (x10 ⁵ /mm ³)	3.39 ± 1.4	2.8 ± 0.74	> 0.05
HB A ₂ (%)	5.4		

Discussion

Percentage OTOFT positive as many as 7.76%, the number still within the limits of estimates prevalence of Indonesian ranged 3-11% [9,14]. Thailand has almost the same percentage with Indonesian carrier have averaged between 3-11 %, but in some places there were pockets of the carrier, even with the amount of carrier reaches 40.4% [14].

Sanchaisuriya et al, research in Thailand with a population of 423 pregnant women get a percentage of the carrier as many as 62.4%, when using combination method OTOFT and

DCIP. Most of the research were thalassemia ϵ and thalassemia α , β thalassemia whereas only about 3% [15]. In this study, and several other research when OTOFT negative result should be examined to find thalassemia ϵ , if it was positive be required to confirm these with other examinations including electrophoresis because there more false negatif.

Based on the characteristics of the average laboratory all sample, hemoglobin mean value was 11.05 ± 1.28 g / dL, it shows that the average hemoglobin levels of pregnant women still above 11 g / dL as anemia physiological limits. Group of anemia as many as 42.9% and non anemia 57.1%, with a range from 6.7 g / dL to 14.5 g / dL. The group anemia with positive results in OTOFT method 8 samples (3.6 %), whereas that does not suffer from anemia as many as 9 samples (4.1%). Research Sanchaisuriya et al, average gain in pregnant women of 12.2 ± 1.1 g / dL that higher than limit normal women with pregnancy (11.0 g/dL) [15].

Other tests that mean value MCV, MCH, the number of erythrocytes, and the number of platelets when looking at the average of all the research subject was still within the normal range. MCV average of 87.28 ± 6.32 fL still above the normal limit of the nature of the suspect was 80 fl. Likewise with the average value of 28.2 ± 2.59 pg MCH was still above the normal value of 27 pg. Sanchaisuriya get an average MCV and MCH in pregnant women respectively 87.9 ± 5.0 fl and 29.9 ± 2.1 pg [15].

Fragile erythrocytes cause the formation of erythrocytes increased. It was seen that the average number of erythrocytes greater than $4.106 / \text{mm}^3$. When compared with the average overall number of erythrocytes on examination OTOFT positive slightly increase, at $4.13.106 / \text{mm}^3$. while results Sanchaisuriya research normal population of pregnant women at $4.1 \pm 0.4 106 / \text{mm}^3$ [15].

Results electrophoresis on Table 2 show HbA2 $>3.5\%$. A thalassemia trait carrier were sometimes no had abnormalities on laboratory result such as hemoglobin level, MCV, MCH and HbA2 normal value but the osmotic fragility test showed positive results. On examination results HbA2 value $\geq 3.5\%$ can diagnosis as a carrier of thalassemia trait possibility of type β , whereas when HbA2 $<3.5\%$ require reconfirmation other tests such as checks HbH inclusion bodies in the blood or DNA analysis. Setianingsih research shows that 32% of patients with thalassemia is the type of α thalassemia. The remaining 68% non- α thalassemia including β thalassemia. In setianingsih study, based HbA2 with values $<3.5\%$ suggests the possibility of

α thalassemia as much as 23.5%, while the rest of the examination HbA2 $\geq 3.5\%$ non- α thalassemia including β thalassemia [9].

In the Table above showed that in the group with OTOFT investigation had average lower value on MCV and MCH, while the number of erythrocytes and platelets higher than negative group OTOFT examination. MCV laboratory results in positive OTOFT inspection group has an average value of 82.64 ± 8.66 fL. When compared with the limits of suspicion was 80 fl, the value was still above level normal but based on statistical calculations there is a significant difference between group of positive and negative OTOFT examination. While the MCH had average examination results in the group of positive OTOFT below normal value (<27 pg), and statistically significant differences between the two groups.

OTOFT platelet counts in the positive group was higher than the negative OTOFT group of about 21%. Eldor & Rachmilewitz research and obtain an increase in the number of platelets and coagulation activity in patients with thalassemia major or intermedia as well as patients with abnormal hemoglobin E [16]. Various theories revealed particularly due to reduced platelet life span of approximately 50% of the platelets in a normal person. Shortened life span of platelets in patients who underwent splenectomy reached 71% of cases, whereas that 35% was not done splenectomy. Increased platelet activation in chronic confirmed by research using flow cytometric tools [18]. Number of platelets in this study despite a higher value on the positive OTOFT groups was statistically not significant difference in both groups.

Carrier alpha Thalassemia sometimes no have laboratory abnormalities such as hemoglobin level, MCV, MCH and HbA2 normal value but the osmotic fragility test showed positive results. HbA2 value $\geq 3.5\%$ can be established as a carrier thalassemia possibility type β , whereas when HbA2 $<3.5\%$ require reconfirmation other tests such as check blood HbH inclusion bodies or DNA analysis because thalassemia α can not exclude yet. This study was needed other examination method like DNA analysis to established diagnosed carrier of the α -thalassemia. Mean value HbA2 all samples 5.4% [17].

Combination of both the OTOFT and the DCIP test as a screening tool for hemoglobin disorders in pregnant women was an effective method and should be used in the antenatal clinic. Study demonstrated the sensitivity, specificity, positive and negative predictive value

for Hb E disorders screened by the DCIP test to be 100%, 97.2%, 94.4% and 100%, respectively. For beta thalassemia disorder screened by the OTOFF test, the sensitivity, specificity, positive and negative predictive value were 100%, 83.7%, 10.5% and 100%, respectively. Evaluation for combining both methods as screening tools for all hemoglobin disorders revealed a sensitivity, specificity, positive and negative predictive value of 100%, 97.1%, 94.9% and 100%, respectively [18].

Conclusion

Population of suspected carrier thalassemia was 7.76%. Combination MCV, MCH value and OTOFT method can use as simple screening tool for detection thalassemia trait in primary level.

References

1. Cunningham FG LK, Bloom SL, Spong CY, Dashe JS, Hoffman BL. Williams Obstetrics. 24th edition. New York: McGraw Hill; 2014.
2. Renzo Galanello R, Raff A. Review Beta-thalassemia. Orphanet J Rare Dis. 2010;5:11
3. Sarnaik SA. Thalassemia and related hemoglobinopathies. Indian J Pediatr. 2005;72(4):319-24.
4. Forget B, Cohen A. Thalassemia syndrome. In: Hoffman R, Benz E, Shattil S, Furie B, Cohen H, McGlave P, eds, Hematology Basic Principles and Practice. 4 edition. Philadelphia: Elsevier; 2005;557-89.
5. Moghaddam ZK, Bayat N, Valaei A, Kordafshari A, Zarbakhsh B, Zeinali S, Karimipoor M. Co-inheritance of α -and β -thalassemia: challenges in prenatal diagnosis of thalassemia. IJBC. 2012;2:81-84.
6. Bianchi N, Borgatti M, Gambari R. Gene silencing in thalassemia. Minerva Biotec. 2008;20:51-5.
7. Cohen A, Galanello R, Pennel D, Cunningham M, Vichinsky E. Thalassemia. Hematology Am Soc Hematol Educ Program. 2004:14-34.
8. Forget B, Cohen A. Thalassemia syndrome. In: Hoffman R, Benz E, Shattil S, Furie B, Cohen H, McGlave P, eds, Hematology Basic Principles and Practice. 4 edition. Philadelphia: Elsevier; 2005;557-89.
9. Setianingsih I, Harahap A, Nainggolan I. Phenotypes and molecular defect. In: Marzuki, Verhoef, Snippe, eds, Alpha Thalassemia in Indonesia. New York: Kluwer academic. 2003;47-56.
10. Ritu R, Pushpa C. Evaluation of NESTROFT as a screening test for β thalassemia trait. J Advance Researches in Biological Sciences. 2013;5(2):127-30.

11. Tangvarasittichai O, Jeenapongsa R, Sitthiworanan C, Sanguansermisri T. Diagnostic value of combined parameters for α -thalassemia-1 screening in pregnant women. Naresuan University Journal. 2004;12(2):19.
12. Thomas P, Oni L, Alli M, Hilaire J, Smith A, Leavey C, Banarsee R. Antenatal screening for haemoglobinopathies in primary care: a whole system participatory action research project. Br J Gen Pract. 2005;55(515):424-8.
13. Chow J, Phelan L, Bain BJ. Evaluation of Single-Tube Osmotic Fragility as a Screening Test for Thalassemia. Am J Hematol. 2005;79(3):198-201.
14. Iswari S, Williamson R, Marzuki S, Harahap A, Tamam M, Forrest S. Molecular basis of β -Thalassemia in Indonesia: application to Prenatal Diagnosis. Mol Diag. 1998;3:11-20.
15. Sanchaisuriya K, Fucharoen S, Fucharoen G, Ratanasiri T, Sanchaisuriya P, Changtrakul Y, Ukosanakarn U, Ussawaphark W, Schelp FP. A reliable screening protocol for thalassemia and hemoglobinopathies in pregnancy. Am J Clin Pathol. 2005;123(1):113-8.
16. Eldor A, Rachmilewitz EA. The hypercoable state in thalassemia. Blood J. 2002;99(1):36-43.
17. Taher AT, Otrock ZK, Uthman I, Cappellini MD. Thalassemia and hypercoagulability. Blood Rev. 2008; 22(5): 283-92.
18. Wiwanitkit V, Suwansaksri J, Paritpokee N. Combined one-tube osmotic fragility (OF) test and dichlorophenol-indolphenol (DCIP) test screening for hemoglobin disorders, an experience in 213 Thai pregnant women. Clin Lab. 2002;48(9-10):525-8.