Reticulocytes indices in β thalassemia trait individuals

Sandrine Comparsi Wagner[1]
Allyne Cristina Grando[2]
Simone Martins de Castro[3]

[1]Instituto de Ciências da Saúde, Universidade Feevale, Novo Hamburgo, RS, Brazil
[2]Universidade Feevale, Novo Hamburgo, RS, Brazil
[3]Analysis Clinical Department, Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul – UFRGS, Porto Alegre, RS, Brazil

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Corresponding author:
Sandrine Comparsi Wagner
Universidade Feevale
RS 239, 2755
93352-000 – Novo Hamburgo, RS, Brazil
Phone: 55 51 3586-8800
sandrine@feevale.br

www.rbhh.org or www.scielo.br/rbhh

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The thalassemias are a diverse group of microcytic and hemolytic anemias that are characterized by defective synthesis of one of the globin chains of adult hemoglobin. The β-thalassemias are characterized by a reduced (β^+-thalassemia) or an absence (β^0-thalassemia) of β globin synthesis which leads to imbalanced globin chain production.[1,2] This imbalanced synthesis results in a variable degree of anemia which stimulates erythropoietin production, leading to proliferation and expansion of the bone marrow. The β-thalassemias have a considerable phenotypic variation depending on multiple factors, which include the nature of the mutation involved. This leads to a wide range of presentations from profound anemia (requiring lifelong blood transfusions – β-thalassemia major) to extremely mild anemia (β-thalassemia trait).[2]

Reticulocytes are juvenile red blood cells; they contain remnants of the ribosomal ribonucleic acid which is present in large amounts in the cytoplasm of the nucleated precursors from which they are derived. The number of reticulocytes in the peripheral blood is a fairly accurate reflection of erythropoietic activity assuming that reticulocytes are released from the bone marrow after the 'normal' time, and that they remain in circulation for the 'normal' period of time.[3]

A total of 152 unrelated adults were included in this study: thirty with the ß-thalassemia trait diagnosed by high-performance liquid chromatography (HPLC-Variant, Bio-Rad, Milan, Italy)[4] with sequencing of the HBB gene using the primers described by Kimura[5] and Miranda[6] and 122 individuals recruited during their routine blood counts at the Pharmacy School Laboratory of the Universidade Federal do Rio Grande do Sul. The Ethics Committee of Hospital de Clínicas de Porto Alegre, Rio Grande do Sul approved the study protocol.

Peripheral blood was collected using EDTA as anticoagulant. Hematological and reticulocyte data were obtained in an automated cell counter - Sysmex SE9500 (Sysmex, Kobe, Japan). Table 1 shows the hematological indices for β-thalassemic trait and control individuals.

Individuals with the β-thalassemic trait presented with significantly higher levels (p-value < 0.05) of the following variables compared to controls: reticulocytes (percentage and number), medium fluorescence reticulocytes, high fluorescence reticulocytes and immature reticulocyte fraction. These results are in agreement with those reported by Noronha & Grotto with the exception of the immature reticulocyte fraction, where no

Table 1 - Mean and reference ranges for hematology laboratory values in the Municipal Laboratory of Curitiba, PR

	Mean (range)		p-value t-test
	Women	Men	
Red blood cells (x 10^{12}/L)	4.7 (4.0 - 5.4)	5.2 (4.3 - 6.1)	<0.05*
Hemoglobin (g/L)	136.2 (118 - 154)	152.8 (127 - 177)	<0.05*
Hematocrit (L/L)	0.41 (0.35 - 0.46)	0.45 (0.38 - 0.52)	<0.05*
Mean cell volume (fL)	87.3 (78.0 - 95.1)	87.9 (78.0 - 97.2)	
Mean cell hemoglobin (pg)	29.3 (25.6 - 32.1)	29.6 (26.1 - 32.7)	<0.05
Mean cell hemoglobin concentration (g/L)	335.8 (319 - 354)	336.9 (322 - 354)	<0.05
Red cell distribution width (%)	13.7 (11.8 - 16.7)	13.8 (12.0 - 16.3)	<0.05
White blood cells (x 10^9/L)	6.7 (3.84 - 10.4)	6.7 (3.9 - 10.9)	**
Eosinophils (%)	3.5 (0 - 11)	4.3 (1 - 13)	<0.05
Eosinophils (x 10^6/L)	228.5 (56 - 682)	284.6 (65 - 940)	<0.05
Basophils (%)	0.5 (0 - 1)	0.6 (0 - 2)	<0.05
Basophils (x 10^6/L)	29.6 (0 - 99)	41.7 (0 - 125)	<0.05
Lymphocytes (%)	33.2 (21 - 48)	33.8 (19 - 49)	
Lymphocytes (x 10^6/L)	2175.3 (1157 - 3500)	2223.2 (1265 - 3648)	**
Monocytes (%)	6.9 (4 - 11)	7.5 (3 - 12)	<0.05
Monocytes (x 10^6/L)	455.1 (208 - 807)	503.2 (192 -968)	
Neutrophils (%)	56 (40 - 70)	53.8 (35 - 69)	
Neutrophils (x 10^6/L)	3777.3 (1804 - 6460)	3762.7 (1728 - 6820)	
Platelets (x 10^9/L)	284.1 (175 - 421)	258.6 (163 - 399)	<0.05**

* - normal; ** - log-normal distribution evaluated by the Shapiro-Wilks test;

statistical difference was seen between the ß-thalassemic trait and control group.[7] In this study no significant difference was found between the groups for low fluorescence reticulocytes.

The reticulocyte count is used as an indicator of the erythropoietic activity of bone marrow in different anemias.[7] Manual techniques (such as supravital staining) have great inter- and intra-observer variability and often the results are inaccurate. Automated cell counting has overcome this limitation. The availability of reticulocyte maturation indices, based on the measurement of RNA content extends the clinical utility of reticulocyte determination.

References

1. Kazazian HH Jr. The thalassemia syndromes: molecular basis and prenatal diagnosis in 1990. Semin Hematol. 1990;27(3):209-28.
2. Weatherall DJ. The thalassaemias. BMJ. 1997;314(7095):1675-8.
3. Lewis SM, Bain BJ, Bates I. Dacie and Lewis: Practical Haematology. 9 ed. London: Churchill Livingstone; 2001.
4. Clarke GM, Higgins TN. Laboratory investigation of hemoglobinopathies and thalassemias: review and update. Clin Chem. 2000;46(8 Pt 2):1284-90.
5. Kimura EM, Grignoli CR, Pinheiro VR, Costa FF, Sonati MF. Thalassemia intermedia as a result of heterozygosis for beta 0-thalassemia and alpha alpha alpha anti-3,7 genotype in a Brazilian patient. Braz J Med Biol Res. 2003;36(6):699-701.
6. Miranda S, Fonseca S, Figueiredo M, Yamamoto M, Grotto H, Saad S, et al. Hb Köln [a2b2 98 (FG) Val-Met] identified by DNA analysis in a Brazilian family. Braz J Gen. 1997;20(4):745-8.
7. Noronha JF, Grotto HZ. Measurement of reticulocyte and red blood cell indices in patients with iron deficiency anemia and beta-thalassemia minor. Clin Chem Lab Med. 2005;43(2):195-7.

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