Bulletin of the *Transilvania* University of Braşov
Series VI: Medical Sciences • Vol. 6 (55) No. 2 - 2013

CLINICAL AND LABORATORY PATTERNS OF HEREDITARY HAEMOLYTIC ANEMIAS IN CHILDREN FROM CENTRAL REGION OF ROMANIA

A. HORVATH [1] M.D. BAGHIU [2]
M. CHINCESAN[2]

Abstract: *Structural defects of the erythrocyte wall, enzyme defects and haemoglobinopathies may cause intrinsic haemolysis. We assessed the clinical and laboratory patterns of 44 patients with hereditary haemolytic anemias. Hereditary sphaerocytosis (HS) and thalassaemia minor and intermedia were equally represented with a mild male predominance in each group. Patients suffering from HS showed more severe clinical symptoms and laboratory changes than patients with thalassaemia minor and intermedia. HS represent a more prominent health care issue in our region compared to the minor forms of thalassaemia encountered in our population.*

Key words: *hereditary sphaerocytosis, thalassaemia minor and intermedia.*

1. Introduction

Hereditary haemolytic anemias are not uncommon in pediatric patients. Structural disturbances in erythrocyte wall development, enzyme defects and defective haemoglobin synthesis or structure are the three categories of intrinsic haemolysis. Haemoglobinopathies are more common in Africa (sickle cell disorders), and Asia (thalassaemias) (Thal) [17], whereas hereditary sphaerocytosis (HS) is the most common inherited anaemia in northern Europe [16] with 0, 02% incidence. Both anemias may show a variety of clinical severity, from asymptomatic cases to severe haemolysis. [20]. Even asymptomatic hereditary anemias are of concern, regarding excessive chronic iron overload with secondary organ malfunctions, and genetic counselling. [5].

HS shows autosomal dominant inheritance in 75% and autosomal recessive inheritance or de novo mutations in 25% of cases and appears in 1:5000 Europeans. Mutated genes encode for defective ankyrin, alpha-spectrin, beta-spectrin, protein 4.2 and band 3 membrane skeletal proteins, which cause loss of membrane lipids and surface area. [2], [7], [11]. Increased neonatal jaundice and later in life the triad of anaemia, jaundice and splenomegaly are the main clinical features of HS. The gold standard for diagnosis in HS is based on: spherocyte morphology of

[1] Department of Pediatrics nr. 2, University of Medicine and Pharmacy, Târgu-Mures.
[2] Department of Pediatrics nr. 1, University of Medicine and Pharmacy, Târgu-Mures.

some red cells on peripheral blood smear, signs of haemolysis and increased osmotic fragility of erythrocytes. Cholelithiasis, haemolytic crisis, erythroblastopenic crisis, folate deficiency, haemochromatosis may complicate HS. Folic acid supplementation and splenectomy are the preferred treatment methods in HS, along with red cell transfusions in aplastic crisis.

Seven percent of the world population is carrier for haemoglobinopathies [17]. Beta-thalassaemias are more likely to appear in individuals of Meditterranean and Asian ancestry. A broad spectrum of clinical severity, from thalassaemia minor with mild hypochromic, microcytic anaemia to beta-thalassaemia major characterized by severe haemolytic anaemia, ineffective erythropoiesis, multi-organ involvement due to iron overload, need for regular red blood cell transfusions and chelation therapy for survival, exists. [8, 9],[19]. The defective gene function in beta-thalassaemia leads to decreased quantitative production of beta-globin chains. The relative excess of alfa-globin chains results in insoluble aggregates causing ineffective erythropoiesis and shortened red cell survival. [14]. Haemoglobin electrophoresis is diagnostic in beta-thalassaemias, with decreased HbA, increased HbA2 and variable Hb F content.

2. Objectives

The purpose of the present paper is to assess the most frequent hereditary anemias in the central region of Romania, based on clinical records of patients admitted to two pediatric clinics from Targu-Mures, Romania. Diagnoses were based on standard laboratory tests; molecular and genetic investigations were not available. We analyzed and compared the clinical and biological features of thalassaemia minor and intermedia and hereditary sphaerocytosis cases.

3. Material and methods

On a total number of 44 patients with hereditary haemolytic anemias we assessed the significant clinical signs and symptoms (jaundice, pallor, splenomegaly) and the haematological and biological changes (reticulocyte number, erythrocyte indices and morphology, red blood cell count, bilirubin, lactate-dehydrogenase, iron, haemoglobin-electrophoresis, osmotic fragility test). Values of biological markers were compared between the two groups of hereditary haemolytic anemias, namely beta-thalassaemia minor and hereditary sphaerocytosis. We used the Mann-Whitney test to detect significant differences.

4. Results

The number of thalassaemia minor patients was almost the same as of those with hereditary sphaerocytosis (20/22), with a slight male preponderance in each group. Thalassemia minor patients were diagnosed at an older age (mean 7, 7 years, limits between 1, 2-15, 5 years) than those with sphaerocytosis (mean 3, 85 years, range 0, 1-9, 9 years). Children with beta-thalassaemia minor were more likely pale (13 pale/7 jaundice) than jaundiced while HS patients were mainly jaundiced (8 pale/12 jaundice). In HS patients splenomegaly appeared more frequently and the size of the spleen was larger (Table 1).

Table 1

Splenomegaly in thalassaemia minor; intermedia and HS

	Thalassaemia minor, intermedia	Hereditary Sphaero-cytosis
Splenomegaly, nr of cases	6 out of 20	13 out of 22
Size of spleen, (cm) below rib border	1-2 cm	2-5 cm

Statistically significant differences between the two groups (Mann-Whitney test, p < 0, 0001) were found in the erythrocyte number (Figure1), which was higher in the thalassaemia group (mean 4, 87 G/L) compared with the HS group (mean 3, 38 G/L), in red blood cell indices and number of reticulocytes.

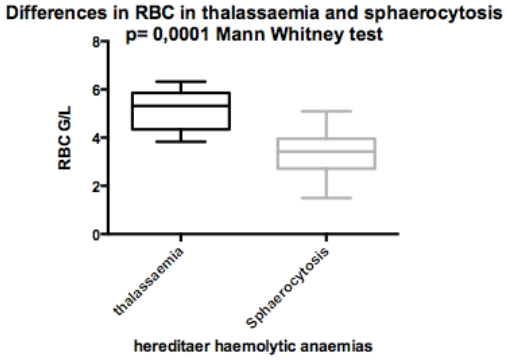

Fig. 1. *RBC in thalassaemia minor;intermedia and HS.*

The mean corpuscular volume (MCV) was significantly lower in thalassaemias (mean 68, 44 fl) than in HS (mean 81, 31 fl) (Figure 2).

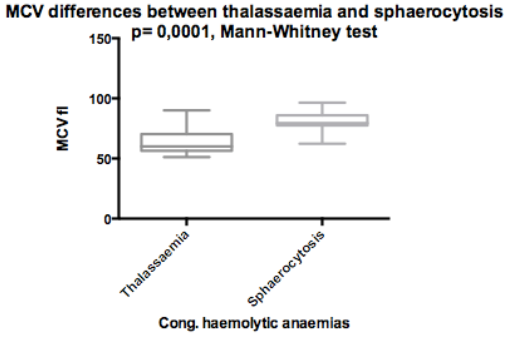

Fig. 2. *MCV in thalassaemia minor; intermedia and HS*

The mean MCHC in thalassaemia minor was 21, 0 and in HS 27, 46. Reticulocytes in thalassaemia minor group ranged between 0, 8-8, 6% (mean 3, 48%) and in sphaerocytosis group between 1, 8-32, 2% (mean 10, 63%) (Figure 3).

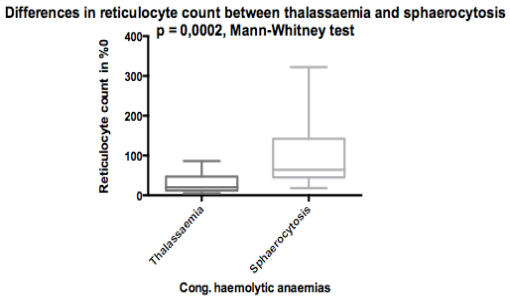

Fig. 3. *Differences in reticulocyte count in thalassaemia minor, intermedia and HS.*

Serum bilirubin level was higher in HS (mean 3, 18 mg/dl, range 0, 58-11, 5 mg/dl) than in thalassaemia minor (mean 1, 18 mg/dl, range 0, 18-3, 49 mg/dl), preponderantly indirect in both groups. Haemoglobin electrophoresis was performed in all thalassaemia patients but only in 3 HS patients. Lower levels of Hgb A along with increased levels of Hgb A2 and Hgb F was the characteristic feature in thalassaemia. Osmotic fragility test of the erythrocytes was performed in all cases with HS but in fewer patients with thalassaemia. Increased osmotic fragility was found in HS, where initial haemolysis appeared at an average of 0, 54% NaCl solution, compared with thalassaemia, where haemolysis started at lower concentration (mean 0, 43% NaCl solution). Variable percentage of sphaerocytes on peripheral blood smear was present in all cases of HS while in thalassaemias anisopoikilocytosis, hypochromia, microcytosis, anulocytes, target-cells, punctate basophilia were more likely to appear. Splenectomy has been performed upon one patient with HS so far after he had been immunized against meningococcus, pneumococcus and Haemophilus influenza. No HS patient has developed gallstones so far. One patient with severe variant of HS presented erythroblastopenic crisis after a viral respiratory infection.

5. Discussions

Elevated MCHC associated with high RDW is very suggestive for HS [11]. The gold standard of diagnosis in HS is the increased osmotic fragility test of the erythrocytes, performed on NaCl- or glycerol- based solutions. However, only 68% of fresh and 81% of incubated blood samples from HS patients showed positive results. Research has been conducted for more reliable tests, so that recently an assay targeting the HS molecular defect has been proposed (the eosin-5'-maleimide- binding test), which has a sensitivity of 93% and specificity of 98% for HS. [3]. Severe depletion in folic acid stores may alter the characteristic haematological features of HS. Such features we have noticed in 2 HS patients who were diagnosed in adolescence and until then they had not received folic acid supplementation. Haemolytic crisis, frequently triggered by viral infection, erythroblastopenic crisis induced by parvovirus B19 infection and deficiency in folic acid are the main hematologic complications of sphaerocytosis. Gallstones may appear in childhood, secondary to increased haemolysis. None of our patients has developed gallstones so far. Splenectomy performed in moderate and severe cases but not before age of 5 years is recommended in HS with some newer recommendations regarding concomitant cholecystectomy [4]. An extended study conducted upon the safety of splenectomy in HS patients showed that splenectomy is a safe though invasive treatment method in HS, with a minimal number of preventable adverse effects. [1].

Heterozygous beta-thalassaemia (thalassaemia trait) is mostly a mild microcytic, hypochromic anemia without clinical symptoms. Features of beta-thalassaemia trait are lower haemoglobin level, MCV < 80 fl, MCHC < 27 pg and HbA2 > 3,5% [12]. A controlled trial performed in Sri Lanka showed that patients with thalassaemia minor may experience symptoms such as fatigue, headache, lethargy, dizziness, exercise intolerance and surprisingly more frequent episodes of pyrexia compared to control group [17]. Splenomegaly does not seem to appear more frequently in these patients. B0 - thalassaemia trait has a lower MCV than B+ - thalassaemia trait [6]. Thalassaemia minor should not be missed for iron deficient anemia, because the extended iron treatments may lead to harmfully increased iron load.

Thalassaemia intermedia (TI) is a clinical diagnosis which denotes those patients whose illness is too severe to be minor and too mild to be a thalassaemia major. In everyday practice, TI diagnosis can be attributed to those thalassaemia patients whose hemoglobin level is less than 9-10 mg/dl and have splenomegaly. Ineffective erythropoiesis, chronic haemolytic anaemia and iron owerload are the features of TI. A broad spectrum of genetic anomalies may be found in TI, moderate or severe cases tend to have the HbE/beta-thalassaemia variant, where the patient inherits the HbE allele from one parent and the beta-thalassaemia allele from the other parent. [15]. Patients with thalassaemia intermedia have increased tissue iron deposition, due to blood transfusions and increased iron absorption. Hepcidin is a 25-amino-acid peptide hormone produced by the liver which inhibits te absorbtion of dietary iron. Intraerythroid factors released through cellular apoptosis associated with ineffective erythropoiesis, inhibit hepcidin production in the liver. [10,13]. Iron overload may be assessed by measuring iron content of liver tissue (> 7 mg Fe/1 g dry weight) or serum ferritin level (> 400-500 µg/l). [18]. Splenectomy and other factors contribute to hypercoagulability

with high rate of thromboembolic events in patients with TI. There are no generally accepted guidelines in the treatment of TI patients, therapy depends on clinical and biological features, and involves transfusion therapy with chelation, splenectomy in individual cases [14].

6. Conclusions

Thalassaemia minor and hereditaer sphaerocytosis showed equal frequency in our patients. Hereditaer sphaerocytosis induced higher level of haemolysis compared to thalassaemia minor patients, resulting in higher reticulocyte count, bilirubin level and increased anemia. The clinical and biological features of the two groups differ. Thalassaemia minor is chracterized by high erythrocyte count, frequently beyond 5 G/L, low haemoglobin and haematocrit, microcytosis, punctatae basophilia, hypochromia, target-cells, decreased HgbA, increased Hgb A2 and variable amount of Hgb F on the haemoglobin electrophoresis. In sphaerocytosis the erythrocyte count is lower, MCV is normal or slightly decreased, reticulocytes are more increased, bilirubin level is higher, on peripheral blood smear the diagnostic microsphaerocytes are omnipresent, the osmotic fragility of erythrocytes is increased. Though HS and thalassaemia minor and intermedia were almost equally represented in our patients, HS showed to be a more prominent health care issue.

References

1. Abdullah, F., Zhang, Y., et al.: *Splenectomy in hereditary spherocytosis: Review of 1, 657 patients and application of the pediatric quality indicators*. In: Pediatr Blood Cancer 52 **(**2009), p. 834-7.
2. Becker, P.S., Lux, S.E.: *Disorders of the red cell membrane skeleton: Hereditary sphaerocytosis and hereditary elliptocytosis.* In: *The Metabolic and Molecular Bases of Inherited Disease,* Scriver, C.S., Beaudet A.L. et al. (eds), New-York, McGraw-Hill Book Co., 1995, p. 3513.
3. Bianchi, P., Fermo, E., et al.: *Diagnostic power of laboratory tests for hereditary sphaerocytosis: a comparison study in 150 patients grouped according to molecular and clinical characteristics*. In: Haematologica 97 (2012), p. 516-23.7
4. Bolton-Maggs, P.H., Langer, J.C., et al.: *Guidelines for the diagnosis and management of hereditary spherocytosis-2001 update.* In: Br J Haematol, 156 (2012), No 1, p. 37-49.
5. Edwards, C.Q., Skolnick, M.H., et al.: *Coincidental nontransfusional iron overload and thalassaemia minor: association with HLA-linked hemochromatosis.* In: Blood 54 (1981), No. 4, p. 844-8.
6. Galanello, R., Origa, R.: *Beta-thalassemia.* In: J Rare Dis, 2010; 5: 11.
7. Gallagher, P, Forget, B.G., et al.: *Disorders of the Erythrocyte Membrane.* In: *Hematology of Infancy and Childhood,* Nathan D, Oski F. (ed). WB Saunders, Philadelphia, USA, 1998, p. 544-665.
8. Gallerani, M., Cicognani, I., et al.: *Average life expectancy of heterozygous beta thalassemic subjects.* In*:* Haematologica, 75 **(**1990) No. 3, p. 224-7.
9. Gallerani, M., Cicognani, I., et al: *Analysis of folate and vitamin B12 in beta thalassemia minor.* In: Riv Eur Sci Med Farmacol 12 **(**1990) No. 4-5, p. 247-50.
10. Ganz, T., Nemeth, E.: *The hepcidin-ferroportin system as a therapeutic target in anemias and iron overload*

disorders. In: Hematology Am Soc Hematol Educ Program. 2011:538-42.
11. Lanzkovski, P.: *Manual of Pediatric Hematology and Oncology*, *Fourth Edition*. Elsevier Academic Press, 2005, p. 136-199.
12. Mazza, U., Saglio, G., et al.: *Clinical and Haematological Data in 254 Cases of Beta-Thalassaemia Trait in Italy*. In: British Journal of Haematology 33 (1976), p. 91–99.
13. Nemeth, E., Ganz, T.: *The role of hepcidin in iron metabolism*. In: Acta hematologica122 (2009), No 2-3, p. 78-86.
14. Nienhuis, A.W., Nathan, D.G.: *Pathophysiology and Clinical Manifestations of the B-Thalassemias*. In: Cold Spring Harb Perspect Med December 2012; 2:a011726.
15. Olivieri, N.F., Murace, G.M.: *Studies in Hemoglobin E beta-thalassaemia*. In: British Journal of Haematology, 141 (2008), p. 388-397.
16. Perrotta, S., Gallagher, P.G., et al.: Hereditary sphaerocytosis. In: Lancet 372 (9647) **(**2008), p. 1411-26.
17. Premawardhena, A., Arambepola, M., et al.: *Is the 𝛽 thalassaemia trait of clinical importance?* In: British Journal of Haematology, 141, (2008), p. 407-410.
18. Taher, A.T., Mussala,m K.M., et al.: *Contemporary Approaches to treatment of beta-thalassemia intermedia.* In: Blood Rev. 26 (suppl.1) (2012), p. 24-7.
19. Taher, A.T., Musallam, K.M., et al.: *Optimal management of 𝛽 thalassaemia intermedia*. In: British Journal of Haematology, 152 (2011), p. 512-523.
20. Lehoczky, D.: *Hemolytikus anaemiak.* In: *Klinikai Hematologia,* Lehoczky, D, Rak, K. (ed). Medicina Konyvkiado Rt. Budapest, HU, 2006, p.133-155.