Review

BETA-THALASSEMIA SYNDROMES, CLINICAL AND LABORATORY APPROACH

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ABSTRACT

The Beta (β) thalassemia syndromes are a heterogeneous group of genetic disorders. The frequency of thalassemia is dependent on the ethnic origins of the patient population. Turkey is located in a geographic area of the world where thalassemia syndromes are common. The incidence rate of β-thalassemia carriers was stated to be 2 per cent in Turkey. Clinical manifestations are diverse and range from asymptomatic hypochromia and microcytosis to profound anemia leading to death in early childhood if untreated. Individuals who are homozygous for the β-thalassemia genes have severe, transfusion-dependent anemia and are said to have β-thalassemia major. Thalassemia intermedia is a condition in which the degree of hemolysis is milder even though the patient may have a deficiency of both β genes. Therefore, thalassemia intermedia is essentially a descriptive term that refers to minimal or no need for transfusions. The presence of one normal gene in the heterozygotes usually leads to enough normal β-globin chain synthesis so that the affected individuals are usually asymptomatic with only a mild anemia. This condition is referred to as β-thalassemia minor or β-thalassemia trait.

In this report clinical and laboratory findings of β-thalassemia syndromes in childhood are reviewed.

Key Words: Beta thalassemia, Childhood, Anemia, Hypocromic microcytic anemia

INTRODUCTION

The Beta (β) thalassemia syndromes are a heterogeneous group of genetic disorders, all characterised by a lack of or decreased synthesis of β-globin chain of Hb A (α_2 β_2) (1).

Thalassemia was not recognized as a clinical entity until 1925, when Thomas Cooley described a syndrome among children of Italian descent characterised by profound anemia, splenomegaly, and bone deformities (2). The first two patients with β-thalassemia major in Turkey were reported in 1941 (3).

Prevalence and Geographic Distribution

The frequency of thalassemia is dependent on the ethnic origins of the patient population (1, 4). The geographic areas in which thalassemia is prevalent closely parallel the regions in which P. falciparum malaria was formerly endemic. Resistance to lethal malarial infections by carriers of thalassemia genes apparently represented a strong selective force that favored their survival in these areas of endemic disease (5-7). About 3% of the world's population (150 million people) carry β thalassemia genes. These genes are particularly prevalent in inhabitants of

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Italy, Greece and Cyprus (8, 9). Turkey is located in a geographic area of the world where thalassemia syndromes are common. The first report about the prevalence of β-thalassemia carriers in Turkey was published in 1971 in which the incidence rate was stated to be 2 per cent (10).Different studies noted a frequency variability, ranging between 3.4 (East Anatolia) and 11 per cent (Western Thrace and Antalya) in Turkey (11, 12).

The pathophysiology

The "globin" part of the hemoglobin predominating in adults (hemoglobin A) is composed of four chains, 2 α- and 2 β-chains (13, 14). The pathophysiologic mechanism in thalassemia is related to an unbalanced synthesis of globin chains or β chains. Normally, the synthesis of α and β chains is balanced, resulting in normal hemoglobin A (α_2 β_2). An unbalanced synthesis of either chain can lead to a failure in the matching of these chains and a defect in hemoglobinization within erythrocytes. In homozygous β-thalassemia, there is an excess of α chains (1, 15). Because of their great instability, free α chains aggregate to form insoluble inclusions in bone marrow erythroid precursors, causing premature destruction of maturing erythroblasts within the marrow (ineffective erythropoiesis) as well as lysis of mature red cells in the spleen (hemolysis)(1).

There are two β genes. Deficiency of one leads to essentially no significant hemolysis (β thalassemia trait/thalassemia minor); deficiency of both genes leads to significant hemolytic anemia (thalassemia major). Thalassemia intermedia is a condition in which the degree of hemolysis is milder even though the patient may have a deficiency of both β genes. Therefore, thalassemia intermedia is essentially a descriptive term that refers to minimal or no need for transfusions (16-19).

Genetics of β-thalassemia

The non-α genes reside on chromosome 11. The β-thalassemias result from the interaction of a large number of different molecular defects in the β-globin genes. Underlying genetic defects include total or partial deletions of globin chain genes, nucleotide substitutions, deletions, or insertions (1, 13, 18), which result in none (β^0) or small amounts (0-20 %) (β^+) of globin chain synthesis. β-Thalassemia trait refers to mutations in one of two β-globin genes.

In contrast to the α-thalassemia syndromes, the β-thalassemias are rarely caused by major structural gene deletions. Most of the β thalassemia syndromes result from one or more nucleotide substitutions or deletions in genes that are otherwise intact. The well-known clinical heterogeneity of the thalassemia syndromes is a reflection of the great heterogeneity of mutations affecting the globin genes. Using techniques such as, restriction endonuclease digestion, gene blotting studies, cloning and sequencing of beta-globin genes, examination of expression of mutant genes in tissue culture cells with the use of plasmid expression vectors, investigators have identified more than 150 different mutations (18). Different studies showed that the molecular basis of β-thalassemia in Turkey is quite heterogeneous and that more than 40 different mutations are responsible for the great variability in clinical expression of this disorder (20-24). The IVS-I-110 (G-A) mutation is the most common β thalassemia defect in Turkey (20, 22).

Clinical Features of β Thalassemia Syndromes

Individuals who are homozygous for the β-thalassemia genes (β^+/β^+ or β^0/β^0) have severe, transfusion-dependent anemia and are said to have β- thalassemia major (Table I). Children with thalassemia major usually develop signs and symptoms of severe anemia in the latter part of the first year of life when normal hemoglobin synthesis becomes increasingly dependent on β-globin. On presentation, affected infants usually have pallor, poor growth and development, and abdominal enlargement (1, 16, 25). In the absence of transfusion therapy, the hemoglobin concentration slowly falls to 3 to 6 g/dl (26). The associated pathophysiologic changes resulting from the subsequent anemia include splenomegaly, which may lead to hypersplenism, osteoporosis, and other skeletal and soft tissue changes associated with an expanded bone marrow, and iron overload resulting from a combination of enhanced gastrointestinal iron absorption and red cell transfusions (27, 28). Prominence of the cheek bones tends to obscure the base of the nose and to expose the upper teeth. Thickening of the cranial bones produces frontal bossing (28). These disturbances of

craniofacial growth constitute the thalassemic facies. The liver, heart, pancreas, pituitary, and other endocrine organs serve as the major sites of excessive iron deposition, which ultimately leads to damage and failure of these organs (1, 16, 26).

The definition of thalassemia intermedia is used to designate a less severe hemolytic anemia. Chronic transfusion therapy is not required except in association with intercurrent illness, and survival into adult life is the rule. These patients usually experience normal growth and development (1). However the phenotype of thalassemia intermedia can be variable ranging from mildly to severely affected patients. Genetically, thalassemia intermedia is homozygous β-thalassemia with alleviating factors such as inheritance of mild β-thalassemia mutations (β^+/β^+), Hb F enhancing factors and co-inheritance of α-thalassemia (29).

The presence of one normal gene in the heterozygotes (β^+/β or β^0/β) usually leads to enough normal β-globin chain synthesis so that the affected individuals are usually asymptomatic with only a mild anemia. This condition is referred to as β- thalassemia minor or β- thalassemia trait (30,31). The syndrome is almost always discovered accidentally during examination for unrelated symptoms or as a consequence of a study designed to characterize better the nature of symptomatic anemia in a family member. Failure to respond to therapy for presumptive iron deficiency anemia often leads to a diagnosis of thalassemia trait. There is no enlargement of the liver or spleen in β-thalassemia trait. Organomegaly suggests a more severe form of thalassemia.

The term thalassemia minima described the situation in individuals who, although obligate carriers of a thalassemia gene, had neither anemia nor abnormal red cell morphology. Each of these clinical phenotypes encompasses a heterogeneous group of genetic disorders including inheritance of heterozygous state for some types of mild β-thalassemia mutations (β^+/β^+). It is undetectable except by inference from family studies (16, 32, 33).

Laboratory Features and Definitive Diagnosis of β Thalassemia Syndromes

In β Thalassemia major, the anemia is severe and first becomes manifest 6 to 9 months after birth, as hemoglobin synthesis switches from Hb F to Hb A. In untransfused patients, hemoglobin levels range between 3 and 6 gm/dl (1, 16, 26). The peripheral blood smear shows severe abnormalities; there is marked anisocytosis (variation in size) with many small and virtually colorless (microcytic, hypochromic) red cells. Abnormal forms, including target cells, stippled red cells, and fragmented red cells, are common. Inclusions representing aggregated α chains are removed by the spleen and hence are not visible in peripheral blood (34). The reticulocyte count is elevated, but because of ineffective erythropoiesis, it is lower than would be predicted from the severity of anemia (26). Variable numbers of poorly hemoglobinized normoblasts are seen in the peripheral blood. The red cells contain either no Hb A at all (β^0/β^0 genotype) or small amounts (β^+/β^+ genotype) (0 to 20%) (16). Hb F is markedly increased (20-100%) and indeed constitutes the major hemoglobin of red cells (1, 16). Hb A_2 levels may be normal, low, or high (2 to 7%). WBC counts are often mildly increased, and there may be mild granulocytic immaturity, sometimes even with myelocytes present (26). Platelets are normal. Increased unconjugated bilirubin levels and other biochemical evidence of hemolysis can be found. The urine often contains increased quantities of urobilin or urobilinogen and may be dark brown because of the presence of dipyrroles and mesobilifuscin (26). The results of ferrokinetic studies are in keeping with ineffective erythropoiesis; plasma iron turnover is increased out of proportion to the increase in erythrocyte iron turnover. Serum iron levels are increased, serum transferrin is often fully saturated, and a non- transferrin-bound iron fraction may be present. The osmotic fragility of red corpuscles is strikingly decreased. The bone marrow is remarkably hypercellular with profound normoblastic hyperplasia. Deficient hemoglobin content of red cell precursors, as well as cytoplasmic inclusions, is apparent (1, 16, 26).

In thalassemia intermedia, parental studies reveal the anemia and microcytosis. However, baseline hemoglobin concentration is higher than thalassemia major with a range of 6 to 9 g/dl

without transfusion (26). Peripheral blood erythrocytes show changes comparable to those of thalassemia major: significant anisocytosis, hypochromia, target cells, basophilic stippling, and numerous nucleated forms. Bone marrow hyperplasia is prominent and inclusions of denatured α chains can be demonstrated in late normoblasts by using supravital stains (34). The hemoglobin electrophoretic pattern is highly variable, a reflection of the heterogeneity of genotypes producing this clinical syndrome. Other laboratory features are derivatives of accelerated heme turnover and iron overload.

Infants destined to develop thalassemia minor are hematologically normal at birth. By 4 months, the hemoglobin concentration, MCV, MCH, Hb A_2 and Hb F levels are all outside normal ranges (35).The postnatal decline in Hb F is delayed, and the normal increase in Hb A_2 is accelerated (36). Anemia is mild or absent. The hemoglobin concentration remains approximately 2 g/dl below normal standards but parallels the normal upward trend during childhood. The red cell count is elevated, and the MCV and MCH values are reduced. The degree of reduction in the MCV is directly related to the degree of reduction in β-globin production (36). The MCVs produced by β^0 mutations are lower than those produced by β^+ mutations. Co-inheritance of a β^0 thalassemia gene and α thalassemia (one or two α-globin gene deletion) normalizes the red cell indices but not the level of Hb A_2 (37, 38). The MCHC is normal or only slightly decreased . In contrast to the minor degree of anemia, morphologic alterations of peripheral blood erythrocytes are prominent. These changes include microcytosis, hypochromia, anisocytosis, poikilocytosis, target cells, and basophilic stippling Nucleated RBCs are not present. The reticulocyte count is frequently elevated. The osmotic resistance of erythrocytes is strikingly increased. Erythrocyte-free protoporphyrin usually is normal, in contrast to elevated levels in iron deficiency (39, 40). The most consistent feature of heterozygous β^0 and β^+ thalassemia is an increase in Hb A_2 (A_2 is not elevated in α thalassemia trait). A_2 is a variant of adult Hb A and is normally present in quantities up to 2.5% or 4%, depending on the method used. In β thalassemia trait, A^2 is elevated to some degree with a maximum of approximately 10%. More than 90% of persons with β-thalassemia trait have diagnostic elevations of Hb A_2 of 3.4-7%. Hemoglobin F is usually normal but can be present in quantities up to 5%. Hemoglobin A_2 cannot be identified on paper electrophoresis, and demonstration or quantitation necessitates cellulose acetate or polyacrylamide gel electrophoresis or resin column methods. Chronic iron deficiency decreases Hb A_2 levels so that iron deficiency coexistent with β thalassemia trait could lead to falsely normal Hb A_2 results (41). Thalassemia minor is easily confused with mild iron deficiency (Table II). Several simple observations distinguish the two conditions. Whereas microcytosis, hypochromia, anisocytosis, and poikilocytosis are minimal in iron deficiency at a hemoglobin concentration of 10 to 11 g/dl, they are conspicuous features of the blood smear in patients with thalassemia trait having comparable levels of hemoglobin. Basophilic stippling and an increase in the icterus index are also prominent, whereas iron deficiency is associated with little or no stippling and with a decrease in serum bilirubin level. Several discriminative functions have been developed to facilitate differentiation of the two disorders on the basis of routine blood counts (42). Patients who have β-thalassemia trait, in contrast to those who have iron deficiency, typically have an increased number of red cells that are smaller than normal, leading to a ratio of the MCV/red cell count per milliliter of less than 13. The ratio in iron deficiency is usually more than 13 (Mentzer index) (43). The coefficient of variation of red cell size is higher in iron deficiency than in thalassemia minor, a derivative readily generated by electronic cell counters (44). Laboratory assessment of iron status can be used to differentiate β thalassemia trait and iron deficiency (Table II). Bone marrow iron is absent in iron deficiency and normal or increased in β-thalassemia trait. Patients with β thalassemia trait may have concurrent chronic iron deficiency and anemia of chronic disease may be concurrent with either condition. The anemia of chronic disease may itself be microcytic and hypochromic in some cases (1).

Silent β thalassemia is inferred in the ostensibly normal parent of a child with thalassemia intermedia if the other parent has high-Hb A_2 thalassemia minor. The MCV and MCH values are normal or only minimally decreased and the hemoglobin pattern is normal. The condition can

be established with certainty only by globin chain synthesis studies, which show an α:β-globin chain ratio of approximately 1.3 (32).

Screening for β-thalassemia

Ideally, thalassemia carriers should be detected as part of a screening programme, such as premarital screening in Cyprus (9) and Sardinia (45), or during early pregnancy in most Mediterranean countries. This allows screening of the partner and if the couple are both carriers then prenatal diagnosis will be possible. In particular, the finding of a non–iron-responsive microcytic anemia in the pregnant woman should prompt a careful evaluation for thalassemia trait that, if present, should lead to a similar evaluation of the father. This approach permits the family to consider in utero diagnosis. Fetus of heterozygote parental pairs can be screened for homozygous thalassemia in utero employing DNA analysis of a chorionic villus biopsy at 8-10 weeks or amniotic fluid cells from amniocentesis at 16-18 weeks (46, 47). The emphasis is prevention of an affected birth by giving the parental pair the choice of terminating an affected pregnancy, and this can only be done by early detection of the carriers. A previous study dealing with prenatal diagnosis of β thalassemia in Turkey has indicated that prenatal diagnosis is feasible in Turkey when early methods of fetal sampling are combined with the advent of PCR-based techniques (24).

Table I: Clinical and Hematologic Features of the β Thalassemia Syndromes

Nomenclature	β Thalassemia Major/ Homozygous β Thalassemia	β Thalassemia Intermedia	β Thalassemia Minor/ Heterozygous β Thalassemia
Genetics	β^0/β^0 β^+/β^+ β^0/β^+	Homozygous β-thal. inheritance of β^+/β^+ mutation, Coinheritance of α thal. Hb F enhancing factors	β^0/β β^+/β
Clinic	Severe anemia Requires blood transfusions regularly	Moderately severe anemia, does not require regular blood transfusions	Asymptomatic child with mild or absent anemia
Hematologic features	Normoblastemia Microcytosis, Hypochromia Anisocytosis Poikilocytosis Basophilic stippling Fragmented red cells	Normoblastemia Microcytosis, Hypochromia Anisocytosis Poikilocytosis Basophilic stippling Fragmented red cells	Microcytosis, Hypochromia Anisocytosis Poikilocytosis Basophilic stippling
Anemia (Hb) (g/dl)	3-6	6-9	9-10

β^0: Mutations that lead to a decreased level of normal β-chain production
β^+: Mutations that lead to absence of normal β-chain production
Thal.: Thalessemia

Table II: Differential Diagnosis of Iron deficiency and Beta Thalassemia Trait

	Iron deficiency	Beta Thalassemia Trait
MCV	Low	Very Low
Serum iron	Decreased	Normal
TIBC	Increased	Normal
Trans. Sat.	Decreased	Normal
FEP	Increased	Normal
Serum Ferritin	Decreased	Normal
Hb A_2	Normal	Increased

MCV: Mean Corpuscular Volume, TIBC: Total Iron Binding Capacity,
Trans. Sat: Transferrin iron saturation, FEP: Free erythrocyte porphyrin
Hb: Hemoglobin

CONCLUSION

Appropriate screening and subsequent diagnostic testing will allow the family physician to diagnose most cases of anemia in children . If there is a question about the diagnosis, the genetics, appropriate counseling, or subsequent evaluation, children suspected of having β-thalassemia should be referred to a pediatric hematologist. These would include patients whose anemia is more severe than would be expected; they may have a confounding second

reason for anemia or they may have β-thalassemia intermedia, β-thalassemia major, which present more complex diagnostic and therapeutic problems.

Although there are excellent treatment modalities today, there is no definitive cure for β-thalassemia yet with the exception of bone marrow transplantation, which is not available to most patients. Until novel therapies, such as Hb F reactivation and gene therapy becomes a reality, the only approach to the control of haemoglobinopathies is prevention. Avoidance of consanguineous marriage, which is 21.21% in Turkey (48), will also help to significantly decrease the frequency of affected births. The most important challenge for the eradication of the haemoglobinopathies in Turkey is the organization of a national genetic preventive programme for the eradication of β-thalassemia, like those going on in several high-risk areas of the Mediterranean basin such as Cyprus and Sardinia. This could be performed either as premarital screening of reproductive couples (49) or by education of the population at risk and their physicians.

REFERENCES

1. *Edward JB, Patricia JVG. Thalassemia syndromes. In: Miller DR, ed. Blood Diseases of Infancy and Childhood. 7th ed. St. Louis, MO: Mosby-Year Book Inc., 1990:460-498.*
2. *Cooley TB, Lee P. Series of cases of splenomegaly in children with anemia and peculiar bone changes. Trans Am Pediatr Soc 1925;37:29. (citation)*
3. *Aksoy M. The history of beta-thalassemia in Turkey. Turk J Pediatr 1991;33:195-197.*
4. *Chernoff A. The distribution of the thalassemia gene: A historical review. Blood 1959;14:899. (citation)*
5. *Siniscalco M. Population genetics of haemoglobin variants, thalassaemia and glucose-6-phosphate dehydrogenase deficiency, with particular reference to the malaria hypothesis. Bull WHO 1966;34:379.*
6. *Nurse G. Iron, the thalassaemias, and malaria. Lancet 1979;2:938-940.*
7. *Nagel RL, Roth EF Jr. Malaria and red cell genetic defects. Blood 1989;74:1213-1221.*
8. *Barrai I, Rosito A, Cappellozza G. Beta-thalassemia in the Po Delta: selection, geography, and population structure. Am J Hum Genet 1984;36:1121-1134.*
9. *Angastiniotis M, Kyriakidou S, Hadjiminas M. The Cyprus thalassemia control program. Birth Defects Orig Artic Ser 1988;23:417-432.*
10. *Cavdar AO, Arcasoy A.The incidence of -thalassemia and abnormal hemoglobins in Turkey. Acta Haematol 1971;45:312-318.*
11. *Bircan I, Sisli S, Guven A, et al. Hemoglobinopathies in the district of Antalya, Turkey. Pediatr Hematol Oncol 1993;10:289-291.*
12. *Kocak R, Alparslan ZN, Agridag G, Baslamisli F, Aksungur PD, Koltas S. The frequency of anaemia, iron deficiency, hemoglobin S and beta thalassemia in the south of Turkey. Eur J Epidemiol 1995;11:181-184.*
13. *Nathan DG, Gunn RB. Thalassemia: the consequences of unbalanced hemoglobin synthesis. Am J Med 1966;41:815-830.*
14. *Weatherall DJ, Clegg JB, Naughton MA. Globin synthesis in thalassaemia: an in vitro study. Nature 1965;208:1061-1065.*
15. *Joshi W, Leb L, Piotrowski J, Fortier N, Snyder LM. Increased sensitivity of isolated alpha subunits of normal human hemoglobin to oxidative damage and crosslinkage with spectrin. J Lab Clin Med 1983;102:46-52.*
16. *McDonogh KT, Nienhuis AW. The thalassemias. In: Nathan DG, Oski FA, eds. Hematology of infancy and childhood. Philadelphia: W. B. Saunders Company, 1993:783-879.*
17. *Orkin S. Molecular analysis of human disease: dissection of beta-thalassemia. Clin Res 1984;32:329-333.*
18. *Kazazian H. The thalassemia syndromes: molecular basis and prenatal diagnosis in 1990. Semin Hematol 1990;27:209-228.*
19. *Nienhuis AW, Anagnou NP, Ley TJ. Advances in thalassemia research. Blood 1984;63:738-758.*
20. *Tadmouri GO, Tuzmen S, Ozcelik H. Molecular and population genetic analyses of beta-thalassemia in Turkey. Am J Hematol 1998;57:215-220.*
21. *Atalay EO, Cirakoglu B, Dincol G. Regional distributions of beta-thalassemia mutations in Turkey. Int J Hematol 1993;57:207-211.*
22. *Basak AN, Ozcelik H, Ozer A, Tolun A, Kirdar B. Molecular characteristic of Turkish patients with beta-thalassaemia. Doga-Tr.J. of Medical Sciences 1991;15:426-434.*
23. *Basak AN, Ozcelik H, Ozer A et al. The moleculer basis of β-thalassemia in Turkey. Hum Genet 1992;89:315-318.*

24. Sukru T, Tadmouri GO, Ozer A, et al. Prenatal diagnosis of β-thalassemia and sickle cell anaemia in Turkey. Prenat Diag 1996;16:252-258.

25. Galanello R, Cossu P, Pirastu M, Cao A. Clinical presentation of thalassemia major due to homozygous beta (0)-thalassemia. Nouv Rev Fr Hematol 1981;23:101-106.

26. Lukens JN. The thalassemias and related disorders: quantitative disorders of hemoglobin synthesis. In: Lee GR, ed. Lee: Wintrobe's Clinical Hematology. Baltimore: Lippincott Williams & Wilkins,1999:1405-1448.

27. Lawson JP, Ablow RC, Pearson HA. The ribs in thalassemia. II. The pathogenesis of the changes. Radiology 1981;140:673-679.

28. Rioja L, Girot R, Garabedian M, Cournot-Witmer G. Bone disease in children with homozygous beta-thalassemia. Bone Miner 1990;8:69-86.

29. Ratip S, Petrou M, Old JM, Wonke B, Porter JB, Modell B. Relationship between the severity of beta-thalassaemia syndromes and the number of alleviating mutations. Eur J Haematol 1997;58:14-21.

30. Metaxotou-Mavromati AD, Antonopoulou HK, Laskari SS, Tsiarta HK, Ladis VA, Kattamis CA. Developmental changes in hemoglobin F levels during the first two years of life in normal and heterozygous beta-thalassemia infants. Pediatrics 1982;69:734-738.

31. Wood WG, Weatherall DJ, Hart GH, Bennett M, Marsh GW. Hematologic changes and hemoglobin analysis beta thalassemia heterozygotes during the first year of life. Pediatr Res 1982;16:286-289.

32. Schwartz E. The silent carrier of beta thalassemia. N Engl J Med 1969;281:1327-1333.

33. Ristaldi MS, Casula S, Porcu S, Cao A. Normal delta globin gene sequence in carrier of the silent-101 (C-T) beta-thalassemia mutation with normal Hb A2 level. Am J Hematol 2001;67:58.

34. Yataganas X, Fessas P. The pattern of hemoglobin precipitation in thalassemia and its significance. Ann N Y Acad Sci 1969;165:270-287.

35. Galanello R, Melis MA, Ruggeri R, Cao A. Prospective study of red blood cell indices, hemoglobin A2, and hemoglobin F in infants heterozygous for Beta-thalassemia. J Pediatr 1981;99:105-108.

36. Rund D, Filon D, Strauss N, Rachmilewitz EA, Oppenheim A. Mean corpuscular volume of heterozygotes for beta-thalassemia correlates with the severity of mutations. Blood. 1992;79:238-243.

37. Kanavakis E, Wainscoat JS, Wood WG, et al. The interaction of alpha thalassaemia with heterozygous beta thalassaemia. Br J Haematol 1982;52:465-473.

38. Melis MA, Pirastu M, Galanello R, Furbetta M, Tuveri T, Cao A. Phenotypic effect of heterozygous alpha and beta 0-thalassemia interaction. Blood 1983;62:226-229.

39. Lyberatos C, Chalevelakis G, Platis A, Stathakis N, Panani A, Gardikas C. Erythrocyte content of free protoporphyrin in thalassaemic syndromes. Acta Haematol 1972;47:164-167.

40. Stockman JA 3rd, Weiner LS, Simon GE, Stuart MJ, Oski FA. The measurement of free erythrocyte porphyrin (FEP) as a simple means of distinguishing iron deficiency from beta-thalassemia trait in subjects with microcytosis. J Lab Clin Med 1975;85:113-119.

41. Kattamis C, Lagos P, Metaxotou-Mavromati A, Matsaniotis N. Serum iron and unsaturated iron-binding capacity in the -thalassaemia trait: their relation to the levels of haemoglobins A, A 2 , and F. J Med Genet 1972;9:154-159.

42. England JM, Fraser PM. Differentiation of iron deficiency from thalassaemia trait by routine blood-count. Lancet 1973;1:449-452.

43. Mentzer WC Jr. Differentiation of iron deficiency from thalassaemia trait. Lancet 1973;1:882.

44. Bessman JD, Feinstein DI. Quantitative anisocytosis as a discriminant between iron deficiency and thalassemia minor. Blood 1979;53:288-293.

45. Cao A, Rosatelli C, Pirastu M, Galanello R. Thalassemias in Sardinia: molecular pathology, phenotype-genotype correlation, and prevention. Am J Pediatr Hematol Oncol. 1991;13:179-188.

46. Schoen EJ, Marks SM, Clemons MM, Bachman RP. Comparing prenatal and neonatal diagnosis of hemoglobinopathies. Pediatrics. 1993;92:354-357.

47. Ravel R. Depletion Anemia. In: Ravel: Clinical Laboratory Medicine. St. Louis: Mosby Year Book, 1995:40-55.

48. Başaran N, Sayli BS, Başaran A, Solak M, Artan S, Stevenson JD. Consanguineous marriages in the Turkish population. Clin Genet 1988;34:339-341.

49. Altay C, Yilgor E, Beksac S, Gurgey A. Premarital screening of hemoglobinopathies: a pilot study in Turkey. Hum Hered 1996;46:112-114.