Ph.D. THESIS

Beta- thalassemia trait - epidemiological and clinical aspects in children in Constanta County

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SUMMARY

I. INTRODUCTION

II. STATE OF KNOWLEDGE

1. History of diagnosis and treatment in beta-thalassemic syndromes
2. History of beta-thalassemic syndromes in Romania
3. Ethnic distribution
4. The influence of malaria
5. Inherited thalassemia
6. Classification of the thalassemias
7. Human Hemoglobins: composition and genetics
8. Molecular diagnosis of beta-thalassemic syndromes
9. Relation between genotip and phenotip of beta-thalassemia
10. Molecular genetic tests of beta-thalassemia
11. Pathophysiology of the beta-thalassemic syndromes
12. Diagnosis of the beta-thalassemic syndromes
13. Treatment
14. Genetic counseling and prenatal diagnosis

III. PERSONAL CONTRIBUTION

A. Introduction
B. Objectives
C. Materials
D. Methods
E. Results and discussions
F. Conclusive discussions
G. Clinical cases
H. Conclusions
I. References
J. Annexes

Key words: beta-thalassemia trait, children, epidemiology, clinical and hematologic aspects
INTRODUCTION

The thalassemias are a heterogeneous group of inherited anemias caused by mutations affecting the synthesis of hemoglobin. Beta thalassemia is most common in persons of Mediterranean, African, and Southeast Asian descent. Thalassemia trait affects 5 to 30 percent of persons in these ethnic groups. Scientists and public health officials predict that thalassemia will become a worldwide issue in the next century. Beta-thalassemic syndromes are frequent in Romania, and the south-east part of our country seems to be the most affected by this kind of genetic abnormality.

The paper “Beta-thalassemia trait - epidemiological and clinical aspects in children in Constanta” consists in 197 pages: 66 pages about the state of knowledge in beta-thalassemic syndromes and 131 pages of personal contribution.

The first part of the thesis, structured in 14 chapters, focuses on the state of the art date regarding beta-thalassemic syndromes: Historical data, Epidemiology, Classification of the thalassemias, Hemoglobin – composition and genetics, Physiopathology, Clinical and laboratory findings in beta-thalassemic syndromes, Treatment. The information is presented using 17 figures and 13 tables.

The main historical data about beta-thalassemic syndromes are systematically displayed, in terms of international and Romanian chronology. The historical aspects are followed by the theoretical part of the thesis describing the main data regarding thalassemia: histological aspects, hemoglobin genetics and classification, clinical findings, laboratory data and therapeutic intervention.

The second part of the paper – the personal contribution - represents two thirds of the thesis, containing 98 tables and 65 figures. There are eight chapters: Objectives, Materials, Methods, Results and discussions, Conclusive discussions, Conclusions, References and Annexes.

The main goals of the work were:
- to make an epidemiological study regarding thalassemia trait in children from Constanta county
- a better understanding of clinical and hematological aspects of thalassemia trait in children
- comparative clinical and laboratory study between ethnical groups diagnosed with beta thalassemia trait
- comparative laboratory study between children with thalassemia trait and children diagnosed with iron deficiency anemia

Material and methods An observational retrospective and prospective study was done on 204 patients diagnosed with thalassemia trait in the Pediatric Department of the County Hospital of Constanta from 1999-2008.

I determined the frequency and the prevalence of the thalassemia trait of the study group, the geographical distribution, clinical findings and hematologic parameters analyses. The data were introduced into electronic system (Microsoft Access 2007) and were statistically analyzed.

The cases were divided in two groups: the retrospective study (41 cases) and the prospective study (163 cases). According with hemoglobin electrophoresis I made a statistic comparative hematologic study between three groups of children: L1 group (HbA2>3.5%), L2 group (HbA2>3.5% and HbF elevated), L3
group (HbA2<3.5 % and HbF elevated). There were used some mathematical formulas in order to compare thalassemia trait with iron deficiency anemia.

**Discussions and conclusions:**

The performed study confirms that the beta-thalassemic syndromes are microcytic hipochromic anemias of normal or hypersideremic type with high level presence worldwide and also present in our country.

The cumulative incidence of heterozygote beta-thalassemia during the study performed (1999-2008) in Constanta County was 0.32%. The prospective study incidence was four times higher (0.49%) compared to the retrospective study.

The achieved prevalence for Constanta City was double (0.28%) than the rest of the County (0.13%). Ethnic prevalence study revealed that the highest prevalence was obtained in Greeks (1.43%), followed by Romani people (1.25%) and Turks (0.29%); the lowest prevalence was obtained for the Romanians.

The average diagnosis age for the entire group of children was 8.38+/−5.85 limited from 9 months to 18 years, having its peak frequency at the 7 to 16 years group (31.86%). The statistic analysis of the average diagnosis age of the prospective group compared to the retrospective group showed no significant statistic differences between the two groups (p=0.3086).

The clinical signs in heterozygote beta-thalassemia was mostly unspecific, the pallor (rarely associated with scleral subicterus: 18.62%) being the only constantly evidenced sign. Mild splenomegaly was diagnosed in 15.19% of the cases, especially in L2 group of children, in which hemoglobin electrophoresis showed HbA2>3.5% and slightly increased HbF (36.36%).

The minor thalassemia associated pathology was diverse: unimportant number of children showed low weight (65 cases), associated iron deficiency anemia (40 cases), common rickets (22 cases), allergic rhinitis (23 cases), infantile recurrent wheezing (12 cases), atopic dermatitis (16 cases), asthma (7 cases). Particularly, we discovered within our group of children one case of Wilson disease, one case of Silver-Russel syndrome and one with diabetes mellitus.

In the performed study we obtained p<0.0001 (extremely statistic significant) for patients diagnosed with asthma/infantile recurrent wheezing (19 cases) compared to the number of asthma cases from all hospitalized cases. Hemoglobin-disease detection should be performed for patients having asthma associated with microcytar hypocromic anemia.

Hemoglobin level was slightly lower for the studied group (average hemoglobin level was 10.55+/−0.97); the majority of the cases (85 cases; 41.67%) had the Hb between 10 and 11g/dl; not to be neglected is the percentage of patients with Hb>11g/dl (38.80%) with high occurrence in children over 3 years old and teenagers; normal hemoglobin does not exclude heterozygote thalassemia.

The average number of erythrocytes was 5.57+/−0.56; the majority of the cases (164; 80.39%) had a number of erythrocytes more than 5 mil/cmm; most of the cases of minor thalassemia associated with iron deficiency had the number of erythrocytes between 4.5-5mil/cmm.

The average of MCV value was 61.38+3.99; most of the cases (100 patients; 49.02%) showed values of MCV between 60-65 fl.

The average of MCH value was 20.02+/−1.76; the majority of the cases (140 patients; 73.53%) had the MCH value between 18-22 pg.
The RDW values were normal in the majority of the cases (137; 67.15%). Increased RDW values were encountered especially in cases of iron deficiency anemia associated with minor thalassemia, as well as in the L3 group of patients (with hemoglobin electrophoresis showing normal HbA2 and slightly increased HbF, interpreted as δβ thalassemia).

The correct interpretation of the blood film in a hypochromic anemia can suggest the diagnosis of heterozygote beta-thalassemia; thus, changes of the erythocitary series as hypochromia, microcytosis, anisocytosis, poikilocytosis, “target”-erythrocytes, macrocytosis, erythrocytes with azurophiles granules and tear drop shaped erythrocytes, schistocytosis, ovalocytosis and spherocytosis being present with a frequency that decreases according to the order above, along with a slightly lower hemoglobin.

Depending on the hemoglobin electrophoresis results, the L1 group (increased HbA2) was best represented (78.92%), followed by the L2 group with both HbA2 and HbF increased (16.18%) and the L3 group (4.9%) with normal HbA2 and HbF present (δβ thalassemia).

By statistic comparison analysis of the three groups, we achieved the following results: between groups L1 and L2 there is a significant statistic difference regarding two parameters: hemoglobin and the number of erythrocytes (p<0.005); between groups L1 and L3 there are the most significant statistic differences for the parameters: Hb, MCV, MCH, RDW (p<0.005); between groups L2 and L3 there is a highly significant statistic difference only for RDW (p<0.005). The majority of heterozygote thalassemia associated with iron deficiency cases were diagnosed between 6 months and 3 years and had Hb between 9 and 10 g/dl; we obtained extremely significant statistic differences (p<0.001) for Hb, MCV, RDW and the number of erythrocytes and highly significant statistic differences for MCH (p<0.01) between these patients and those without associated iron deficiency. Furthermore, for the heterozygote thalassemia with associated iron deficiency group, we obtained a much lower percentage of accurate diagnosis (by applying differential formulas) than the group without iron deficiency.

The best indexes that showed the distinction between iron deficiency anemia and minor thalassemia (as Youden criteria) were Mentzer and Ehsani indexes (Youden index=95.11), followed by England index (Youden index=93.23), Shein-Lal index (Youden index=82.70) and Srivastava index (Youden index=81.95). The two indexes (Mentzer and Ehsani) together can provide a better distinction between the two types of anemia, hypochromic and microcytic. Monitoring hematological parameters such as RDWi (Youden index=93.6) and RBC (Youden index=87.96) proved to be useful as well since these parameters obtained maximum Youden indexes in other studies.

The data of the present study allow issuing the hypothesis of the existence of an iceberg phenomenon, assuming that the real incidence and prevalence in Dobrogea is much higher and that these epidemiologic indexes will increase during the next years, pending that medical and research activity, both at primary assistance level (family medicine) and secondary and third level assistance (hospital), will further pursue the discovery of heterozygosity, especially by activities within the outbreak center.

Considering that the beta-thalassemic syndromes represent a public health issue, both by their frequency and by the social and medical problems they arise, it is of outmost importance that primary prophylaxis should be performed in a
correct manner, at least by timely postnatal identification of heterozygotes, useful method for medium occurrence rate of heterozygotism.

The clinical and laboratory study, based on hematological examinations (Hb, Ht, erythocytary indexes, blood film examination and so on) of heterozygote beta-thalassemia cases led to the elaboration of a clinical and hematological profile of the heterozygote beta-thalassemia for an easier diagnosis at lowest ages possible, when primary and secondary prophylaxis will have the best efficiency.

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