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Research Article

Haemoglobinopathy in Slovakia

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Abstract

Objectives: The paper presents results of 27- year epidemiological study of screening and follow-up haemoglobinopathies in Slovakia.

Methods: Between 1993 – 2020, in two research centres in Bratislava and in one centre in Kosice, carriers of beta-thalassaemic genes or other haemoglobinopathies were searched for. Diagnosis was performed by haematologists, whereby the family history was evaluated, together with overall clinical condition, blood count and blood smear, iron and haemolysis parameters, mutations of hereditary haemochromatosis, and haemoglobin electrophoresis testing. Patients with a probability of having a haemoglobinopathy were sent to the research facilities.

Results: 415 patients were genetically examined. In 385 (92.77%), of them heterozygote beta-thalassaemia was confirmed (in 98 families). Five patients were diagnosed for delta, beta-thalassaemia (1.20%), 4 patients (0.97%) for delta, beta-gama1-thalasaemia or persistent hereditary fetal haemoglobin. In total we diagnosed 20 mutations of beta-globin gene. The most frequent mutations were IVS 1.110 (G-A), IVS II-1(G-A) and codon 39. Evidence of haemoglobin S (heterozygote sickle cell anaemia), was also notable in two non-relative children, whose fathers were of African origin, in one patient of Ghana and in one patient from Nigeria. One female patient was followed up for haemoglobin Santa Ana (mutation de novo), one family for haemoglobin Bishopstown and one patient for mutation KLF1 gene.

In our group were 14 patients (3.17%) diagnosed for alpha-thalassaemia.

All patients were heterozygotes, only one female patient from Macedonia was a double heterozygote for beta-thalasaemie.

Clinicaly all of the patients had a minor or intermedia form.

In the years of 2012-2019 we observed 12 pregnant patients with beta-thalasaemie. One of them had multiple pregnancies, all deliveries were without haematological complications.

Conclusions: The study showed that in the west and eastern Slovakia there is a higher number for thalassaemia and other haemoglobinopathies. Mutations are of historical origin or over the past years we have recorded an increase number of mutations from areas with high incidence of haemoglobinopathies. It is necessary to continue in search of pathological gene carriers to avoid serious forms of the disease.

INTRODUCTION

Haemoglobinopathies existed originally mostly in the areas with high incidence of malaria, such as Mediterranean regions, some regions of Africa and South East Asia. Since plasmodia of malaria were unable to survive in the afflicted erythrocytes, the haemoglobin mutations carriers had selective advantage survival and they were able to transfer the mutated genes to future generations. Thanks to this factor, the prevalence of thalassaemia and other haemoglobinopathies was increasing in the formerly presented regions.

In the past, it was assumed that thalassemia and other hemoglobinopathies were almost non-existent in Slovakia in the middle of Europe. However, our territory has historically gone through various periods of wars and waves of emigration. As a result, it seems unlikely that these events would not have left some genetic traces behind in the population.

Several works [1,2], showed the incidence of hemoglobinopathies in the Slovak and the Czech Republic, but in the past no specific study was performed in Slovakia. Therefore, in 1993 we founded a study group at the Slovak Hematological Society for the research of thalassemia and other hemoglobinopathies.

The study center has become the University Hospital Bratislava-Kramare, the National Institute of Pediatric Diseases, Bratislava and the Pasteur University Hospital, Kosice. Dispensary for patients with hemoglobinopathies has been established at the

St. Michal Hospital in Bratislava and we have already published partial results of our study [3,4]. Due to epidemiological situations in Europe and due to ongoing demand from the field practice, we have continued with our epidemiological study. Meanwhile, the awareness of health professionals and patients alike has improved, and new examination methods such as electrophoretic or high performance liquid chromatography (HPLC) (and, most importantly, genetic examinations) have been utilised.

We have not only continued searching for hemoglobinopathies in the domestic population, but in foreigners and in descendants from mixed marriages with foreigners as well. Current results of the study for the period 1993-2020 have been presented.

MATERIALS

Patients with suspected haemoglobinopathies were addresed in the local hematological surgeries or were sent directly to the aformentioned centres.

Criteria for participating in the study were as follows:

-positive family history (e.g. originating from certain ethnic areas);

-objective finding (e.g. icterus, splenomegaly);

-microcytes of erythrocytes in the blood count; MCV (medium volume of ertyhrocytes) below 78fl, possibly MCH (medium size of haemoglobin in the erythrocyte) below 25pg, anaemia was not necessarily present;

-finding in the peripheral blood smear: microcytosis, target erythrocytes, possibly further morphological changes;

-normal or elevated levels of serum iron, particularly ferritine or persistent microcytosis of erythrocytes following treatment by iron preparations;

-positive tests for haemolysis (increased reticulocyte count, reduced levels of haptoglobin, elevated indirect bilirubin, etc.)

METHODS

-parameters of blood counts (reticulocyte count, MCV, MCH) were obtained from normal high-parameter counters of blood cells;

-peripheral blood smears were evaluated microskopically (Pappenheim´s staining);

-examination of parameters of iron, bilirubin and other biochemical parameters was conducted through standard biochemical analysers; haptoglobin test was performed by laser nephelometry;

-electrophoresis of haemoglobin tests was carried out by the acetate celulose sheets method in the aformentioned haematological centres. The values of haemoglobin HbA2 below 3.5% (adults), and foetal haemoglobin HbF below 1.1% were considered normal; higher values for HbA2, possibly for HbF were considered pathological;

-Over the last years, the haemoglobin division was also examined by high performance liquid chromatography (HPLC) method.

Only patients meeting the above mentionad criteria were accepted to participate in the study.

If the results came back positive, we would ask the proband´s relatives to be referred to us; this include ascending, descending and horizontal line of the family.

The genetic diagnostic was conducted by the revers hybridisation method in the Laboratory of Clinical Genetic, Faculty hospital, Nitra; in the Department of Biology, Faculty of Medicine, Palacký University Olomouc and in the Genetic Laboratory Alpha Medical, Košice by SQ and MLPA analysis.

Whereas haemoglobinopathies tend to store iron we wanted to exclude another factor negatively affecting the metabolism of iron. Since 2013 we have also examined the mutations of hereditary haemochromatosis protein (HFE), C282Y, H63D and S65C in patients with the confirmed mutation for haemoglobinopathy.

RESULTS

Genetic examination was performed in 415 patients; of these 25 (6.02%), were foreigners. Beta-thalassemia was found in 385 (92.77%) cases; there were 98 families (at least 2 members considered to be a family), others were individuals. We have diagnosed 20 types of mutations in betaglobin gen. Distribution of beta-globin mutations is shown in Table 1. One woman from Macedonia was double heterozygous for the combination IVS 1-6 (T- C) and IVS 1.110 (G-A). Delta, beta-thalassemia was found in 5 patients (1.20%). Delta,beta,gamma-thalassemia , resp. fetal hemoglobin persistence (HPFH), were found in 4 people (0.97%). Mutation for the hemoglobin variant HbS was found in 4 patients (1.20%); of these 2 were unrelated children whose father was from Africa. The other 2 cases were also of African origin. One patient had the hemoglobin variant Hb Santa Ana (de novo mutation). One child had a mutation in the KLF1 gene. One family had Hb Bishopstown.

We found alpha-thalassemia in 14 patients (3.14%). In 3 patients there was the form of del.3.7; one patient of African origin had homozygous mutation. Other mutations were deletion forms in the region of alpha globin family genes including HBA1/HBA2 and adjacent regions. The results are shown in Table 2. We were looking for the presence of hereditary hemochromatosis. In a group of 70 subjects we found in 37 (52.85%), of them a mutation of the HFE protein, 2 patients were homozygous for C282Y mutation. We also examined MRI (SIR method), for Fe content in dry liver tissue and found increased values in homozygous patients only with hereditary hemochromatosis. Of course, we excluded history of alcohol abuse, hepatopathy or any other disease causing elevated iron levels.

Patients under study had minor or intermediate forms of anemia with lowest hemoglobin levels around 80-90g/l thus requiring no treatment except for vitamins. Parenteral Fe administration was not needed except for a short -term treatment in women with metrorrhagia.

Since 2012 we have provided counselling for 12 pregnant women with hemoglobinopathy. Each partner was also examined and there were found no signs of hemoglobinopathy. Pregnant women with mild to moderate anemia were treated with folic acid

Table 1: Beta-thalasaemie in Slovakia (1993-2020).

Mutations	HGVS Name	Number: pts/fam.	% pts/fam.
IVS I-110 G>A	HBB:c.93-21 G>A	112 / 35	29.09 / 35.71
IVS II-1 G >A	HBB:c.315+1 G >A	79 / 22	20.51 / 22.45
CD 39	HBB:c.118 C >T	55 / 10	14.28 / 10.20
IVS I-6 G>A	HBB:c.92+6 T> C	41 / 10	10.64 / 10.20
IVS I-1 G>A	HBB:c.92+1 G >A	24 / 6	6.23/ 6.12
CD 8 (-AA)	HBB:c.25_26del AA	23 / 4	5.97/ 4.08
IVS II-745 C>G	HBB:c.316-106 C> G	12 / 3	3.11 / 3.06
CD 27 GCC>TCC	HBB:c.82 G> T	11 / 2	2.86 / 2.04
CD 121 G>T	HBB:c.364 G> T	9 / 3	2.34 / 3.06
CD 5 - CT	HBB:c.17_18delCT	4 / 1	1.04 / 1.02
IVS I-5 G> C	HBB:c.92+5 G> C	4 / 2	1.04 / 2.04
CD 44 - C	HBB:c.135delC	2	0.51
CD 58 CCT>CT	HBB:c.176delC	1	0.26
CD 57/58 -C	HBB:c.174_175delC	1	0.26
CD 82/83 -G	HBB:c.251delG	1	0.26
-223 T> C	HBB: c.273 T> C	1	0.26
CD 41/42>CTTT	HBB:c.126_129delCTTT	1	0.26
CD 17 AA>TAG (LysSTOP)		1	0.26
-87 C> G	HBB:c.-137 C> G	1	0.26
5UTR+20C> T	HBB:c.-31 C >T	1	0.26
IVS I-110 G >A + IVS I-6 G> A		1	0.26
∑ 20		∑ 385 / 98	*99.96 / 99.98

Table 2: Other haemoglobinopathies in Slovakia (1993-2020).

Other haemoglobinopathies	Number: pts/family	
Hemoglobín S	4	1 pt. 30% content
Delta,beta-thalassaemie	5	
Delta,beta,gama tal./HPFH	4	
Alfa-thalassaemie		
Alfa tal.[3,7]	3	1 homozygot
Del.HBA1/HBA2 gen	5/1	
Other deletions	6	
Hb variants		
Hb Santa Ana	1	
Hb Bishopstown	1 / 1	
Mutation KLF1 gen	1	

and vitamin B12 parenterally since no one required erythrocyte transfusion. Ten pregnancies had not complicated delivery; one woman had to have a Cesarean section, another woman received transfusion due to postop bleeding and oxytocin allergy. Blood count in all patients returned to baseline after 6 weeks. All couples underwent prenatal and ante-natal counselling, especially when one partner was from a country with a high incidence of hemoglobinopathies.

DISCUSSION

Originally, the incidence of hemoglobinopathies in Europe had been rare but the rate has increased along with changes in the genetic structure due worldwide migration waves.

In the 90's, the WHO drew attention to the problem of hemoglobinopathies and then in 2002 they set-up the Establishment of the European Network for Rare and Congenital Anemias (ENERCA), which focused on detection, diagnosis and treatment of rare anemias as well as on the evaluation of their financial impact on health care systems in Europe. ENERCA, together with other organizations such as the Thalassemia International Federation (TIF), have presented specific screening programs along with recommendations for countries with high and low incidence of hemoglobinopathies, respectively [5-8].

These organizations organize various educational programs for health professionals and laymen alike. For instance the educational program the Renzo Galanello Fellowship, TIF e-academy, THALIA (Thalassemia in Action) project which provides counselling for countries with high numbers of migrants and refugees such as France , Germany, Sweden but also for transit countries such as Greece, Serbia, Austria etc [9] In addition, organizations such as EuroBloodNet (ERN), have dealt with rare diseases including hemoglobinopathies and most recently are involved in activities aimed at the protection of people with

hemoglobinopathies against Covid-19 [10,11]. Furthermore, up to date reports are also provided by various international events e.g. annual hematology congress of the American Society of Hematology (ASH), and the European Society of Hematology (EHA).

Slovakia is among countries with low incidence of hemoglobinopathies yet as our study published in 2012 and 2017 has pointed out. Acquired mutations of historical origin have been transmitted over generations in their heterozygous forms and thus being presented with no major clinical manifestation. Until recently, hemoglobinopathies, therefore, did not attract medical attention. However, events such as the fall of Iron Curtain and great migration from malaria prone regions to Europe have caused a new interest in the study of those latent forms of hemoglobinopathies.

In medical practice we suspect hemoglobinopathy after performing clinical and laboratory exams routinely finding erythrocyte microcytosis with normal values of iron parameters, hemolysis etc. In addition, ELFO hemoglobin and HPLC are utilised yet other methods such as capillary electrophoresis, isoelectric focusing and mass spectrometry are gaining grounds. Nonetheless, the final confirmation is genetic testing which enabled us to diagnose delta-beta-gamma thalassemia and other deletions of the HBB gene, alpha thalassemia and other hemoglobin variants. In this respect we have cooperated for many years with the important genetic center Department of Biology, Faculty of Medicine Palacky University Olomouc, Czech republic.

Our work focuses on the prevention. The problem is that individuals with mild heterozygous forms passing mutated genes on next generations may have led to the occurence of giving a birth to children with severe homozygous forms of hemoglobinopathy. The only way to manage that situation is prevention since based on the type and combination of mutations it is possible to reasonably predict how clinically serious the condition of a child might be and what treatment options might be available. Nowadays there are almost a thousand known mutations for globin genes often intertwined with other risk factors, and therefore, each case should be considered individually. This requires awareness of health professionals, governments, the media, general public with patients organizations to develop and to support adequate screening programs.

CONCLUSION

The changing geopolitical situation in the world has raised a lot of social and health issues. Spread of hemoglobinopathies, namely severe clinical manifestations in Europe might be one of them [12]. In Slovakia, on one hand, we have experienced an increased influx of foreigners, on the other, some of our people are working abroad, so the migration of people often brings about mixed marriages with children who increasingly may have inherited mutations for hemoglobinopathies. Our goal for the future is to define the incidence of hemoglobinopathies, namely in Central Slovakia, since the western and the eastern parts of the country had already been surveyed. We further want to expand genetic testing in order to detect/prevent development of severe forms which represent difficult health, economic and social problems.

Finally, as an EU member, we are obliged to participate in various European programs including the influx of migrants which puts heavy pressure on the health care systems that will be required to deal with many issues, such as taking care of a population segment with an unusual genetic setting. It would be reasonable to give those issues adequate attention because in the near future we could be confronted with hemoglobinopathies that pose a very challenging health care issue even for us.

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