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Molecular diagnostics of thalassemia α in Polish population**

Diagnostyka molekularna talasemii α w polskiej populacji

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Keywords

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Słowa kluczowe

talasemia α, technika ASO, test Alpha-Globin StripAssay®, mutacja -α$^{3.7}$, mutacja --^{FIL}

Summary

Introduction. Thalassemia α is a hereditary haemolytic anaemia, in which mutation in one or more of α globin genes leads to decrease in protein synthesis or complete absence of α globin chains. It is very common in tropical and subtropical populations, however it is now more often described in North America and northern Europe. Molecular diagnostics allows to detect cases of thalassemia α in reliable manner. One of the methods is reverse dot blot or ASO (allele-specific oligonucleotide probes) technique.

Aim. Identification of α thalassemia cases among pediatric patients with undiagnosed haemolytic anaemia.

Material and methods. Pediatric patients with undiagnosed haemolytic anaemia based on clinical, morphological (microcitosis) and biochemical parameters, tested with the use of ASO technique.

Results. Approximately 15% of the patients were α thalassemia cases, with the -α$^{3.7}$ mutation being the most common. We have also found one --^{FIL} mutation, very rare in other than Southeast Asia populations.

Conclusions. This results indicate that thalassemia α may be a cause of hereditary haemolytic anaemia in populations where this disease was considered extremely rare.

Streszczenie

Wstęp. Talasemia α jest wrodzoną niedokrwistością hemolityczną, w której mutacje w jednym lub większej ilości genów α globiny prowadzą do ograniczenia lub braku syntezy białek łańcuchów α globin. Występuje często w rejonach tropikalnych i subtropikalnych, a także jest obecnie opisywana w Ameryce Północnej i w północnej Europie. Diagnostyka molekularna pozwala niezawodnie wykrywać przypadki talasemii α. Jedną z metod jest technika ASO (wykorzystująca allelospecyficzne sondy oligonukleotydowe), wariant metody odwrotny dot-blot.

Cel pracy. Zidentyfikowanie przypadków talasemii α wśród pacjentów pediatrycznych z niezdiagnozowaną niedokrwistością hemolityczną.

Materiał i metody. Pacjenci pediatryczni z niezdiagnozowaną niedokrwistością hemolityczną rozpoznaną na podstawie parametrów klinicznych, morfologicznych (mikrocytozy) i biochemicznych badani metodą ASO.

Wyniki. 15% badanych osób okazało się przypadkami talasemii α, z najczęstszą mutacją -α$^{3.7}$. Znaleziono również jedną mutację --^{FIL}, która występuje bardzo rzadko poza Azją Południowo-Wschodnią.

Wnioski. Uzyskane wyniki wskazują, że talasemia α może być przyczyną wrodzonej niedokrwistości hemolitycznej w populacjach, w których ta choroba uznawana jest za niezwykle rzadką.

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INTRODUCTION

Thalassemias are large group of haemolytic anaemias connected with mutations in one or more globin genes. This leads to decrease in protein synthesis or complete absence of globin chains. The most common are alpha (α) and beta (β) thalassemias with carrier frequency > 1% in all tropical and subtropical populations (1). Alpha thalassemia is the most frequent in Southeast Africa, the Mediterranean area, the Middle East and Africa (2). In some areas the incidence of this disorder is as high as > 40% (Middle Eastern and Indian populations) and > 80% (Papua New Guinea and small populations in north-east India) (2). However, due to accelerated migration and increased percentage of mixed marriages, thalassemias are more often described in different areas, like North America and northern Europe (1, 3-5). Improvement in molecular diagnostics allowed not only to diagnose such cases of the disorder, but also to find mutations in globin genes in indigenous European and American populations.

In human, there are four α globin genes, two on both chromosome 16. Alpha thalassemia mutations remove one of the genes on chromosome (thalassemia α^+), or both of them (thalassemia α^0) (2). Absence of one or two globin genes is usually asymptomatic. Lack of three of them leads to HbH disease, with anaemia of varying degrees. Absence of all α globin genes is connected with haemoglobin Bart's hydrop fetalis, the lethal form of α thalassemia, if the mutations are α^0 type (1, 5).

In Poland, thalassemias were long considered as insignificant in diagnosis of haemolytic anaemias. However, recent studies revealed presence of α and β thalassemia cases in our population (6-8).

AIM

Identification of α thalassemia cases among pediatric patients with undiagnosed haemolytic anaemia.

MATERIAL AND METHODS

We have examined 48 pediatric patients from two pediatric departments with symptoms of haemolytic anaemia based on clinical, morphological and biochemical parameters. All patients have signed the informed consent forms, and the studies were approved by appropriate bioethical committee. The laboratory analyses included measurement of haemoglobin level (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), red blood cell distribution width (RDW), serum iron (Fe) and serum ferritin concentration.

Blood was collected using EDTA as anticoagulant and DNA was isolated with the use of QIAamp DNA Blood Mini Kit (Qiagen, Netherland). The reverse dot blot or ASO (allele-specific oligonucleotide probes) technique was used (Alpha-Globin StripAssay®, ViennaLab, Austria) to analyze mutations in α globin genes. This method allows to identify 21 of the most common mutations underlying α thalassemia.

RESULTS

Forty eight pediatric patients with symptoms of haemolytic anaemia were examined in order to identify α thalassemia cases. The average and the range of biochemical and morphological parameters indicating presence of haemolytic anaemia in examined children are shown in table 1. Thirty one of them were male, and 17 female. The age of the patients ranged from 1.5 to 17 years.

Alpha thalassemia mutations were analyzed with the use of reverse dot blot technique. Of total 48 probes, 32 gave proper results, with correct test controls. Out of 32, 7 tests were positive, and presence of α thalassemia mutations in heterozygous state was detected. Among 7 defects, 6 were $-\alpha^{3.7}$ and one $--^{FIL}$ mutations. An exemplary positive test result is depicted in figure 1.

DISCUSSION

Alpha thalassemia is the most common globin disorder and the most frequent monogenic gene disease (1, 9). It is known that the carrier state of α thalassemia is one of the mechanisms protecting against *Plasmodium* infection and malaria (10), hence the high frequency of this disorder in tropical and subtropical regions (10-80% in different areas) (2). Surprisingly, α thalassemia, when taken into consideration while diagnostics of haemolytic anaemias, is also found in indigenous populations of northern Europe and North America. A screening program in California, implemented in 1998 revealed that α thalassemia becomes growing health problem in USA. Between January 1998 and June 2006, 11.1 cases of α thalassemia per 100,000 persons were reported. Moreover, 406 of them were HbH disease and 5 haemoglobin Bart's hydrop fetalis cases (5). In turn, one of the European studies, showed the highly heterogenous spectrum of α globin mutations among allo- and autochtonous populations in Netherland. Nineteen different mutations (deletions and point mutations) were found among 139 independent chromosomes, with the $-\alpha^{3.7}$ mutation being the most common (4).

In Poland, interest in diagnostics of thalassemias is a novel approach. For years this disorders were considered extremely rare or even absent (6). A significant

Table 1. The average of morphological and biochemical parameters in children with microcitosis suspected of talassemia α

Parameter normal values	Hb 11.0-14.6 g/dl	MCV 76-90 fl	MCH 25.5-32 pg	RDW 11.5-14.5%	Fe 50-175 μg/dl	Ferritin 7-140 ng/ml	HbF 1-2%	HbA2 2.5%
Average patients' values	11.52	68.23	23.13	15.11	77.9	46.61	2.26	2.96

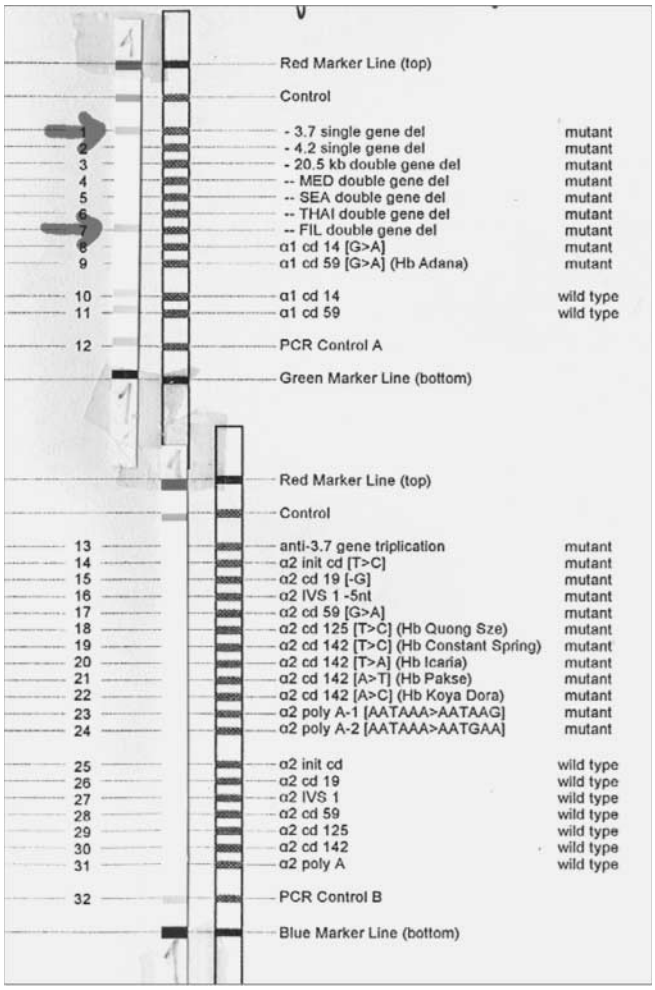

Fig. 1. An example of positive Alpha-Globin StripAssay® result

improvement in molecular diagnostics simplified the search for mutations in globin genes. β thalassemia was described in several studies. In one of them 216 patients were analyzed, and β thalassemia was confirmed by genetic analysis in 173 of them, with two homozygotes and two compound heterozygotes (6). In another study, 21 patients were examined, and 14 different β globin mutations were found, including 3 mutations not reported previously (7).

Alpha thalassemia in our country was not studied so well, and few cases of this disorder have been described. Two pediatric patients with mild haemolytic anaemia were examined to search for α and β thalassemia. They both had the most frequent in European population $-\alpha^{3.7}$ mutation (11). In another study, among 52 children with haemolytic anaemia, 10 cases of β thalassemia and 6 cases of thalassemia α were fund (12). In subsequent patient, a relatively rare case of coexisting hereditary spherocytosis and α thalassemia was described (13).

Our results show that α thalassemia may be a frequent cause of undiagnosed cases of haemolytic anaemia. Of 48 examined pediatric patients with clinical, biochemical and morphological symptoms of anaemia, 7 were diagnosed as α thalassemia cases. The frequency of α thalassemia in examined population could be even higher, because of technical difficulties and lack of the material to repeat the unsuccessful tests.

Although the best strategy to diagnose α thalassemia and the only approach allowing for identification of particular variant of the disease are DNA-based techniques, there are other indicators of α thalassemia. Among them, the most important are globin chains and haemoglobin electrophoresis and α/β globin chain synthesis ratio measurement. Level of HbF and HbA_2 is usually normal, which differs α thalassemia from β thalassemia and haemoglobinopathies (1). Additionally, morphological and biochemical parameters may be of help in diagnosis of α thalassemia. Out of them, the most important are RDW – normal or slightly elevated in thalassemia, symptoms of microcytic hypochromic anaemia (reduced MCV, MCH and Hb) and iron and ferritin levels, normal in α thalassemia and rouling out the iron deficiency anaemia (9, 12, 20). Parameters listed above were very helpful in selecting patients to our analyses. Their results, as shown in table 1, are consistant with parameters characteristic for α thalassemia described in literature.

The most common α globin gene defect is $-\alpha^{3.7}$ mutation. Both α globin genes on chromosome 16 are located within two highly homologous duplication units. The whole region is divided into three homologous segments, X, Y and Z. Mutation $-\alpha^{3.7}$ appears when reciprocal homologous recombination between Z fragments (3.7 kB apart) takes place. That leads to α^{+} thalassemia – lack of one of the α globin genes on chromosome 16 (14, 15). Mutation $-\alpha^{3.7}$ is the most frequent in all examined populations, in some of them covers 80% of all of the α globin deffects (16). It was the most frequent mutation in the Dutch study mentioned above (4), in the large Turkish study – 85 cases of $-\alpha^{3.7}$ mutation among 330 patients (17), in Sicily – 46.94% (18) and Spain – 52.41% (19). Most of the patients in our country described in literature also had $-\alpha^{3.7}$ deffects (11, 13). This observations are consistant with our results. Of 7 cases of α thalassemia, 6 were $-\alpha^{3.7}$ mutation. We have found only one case of another defect, and it was Filipino ($--^{FIL}$) mutation. It is one of the most common α^{0} globin defects, removing both α globin genes from chromosome 16 (20). Additionally, ζ globin gene is removed, which results in lack of embryonic haemoglobin Portland and termination of the pregnancy in homozygous state of the embryo (21). This mutation is the most frequent in Philippines, China and the rest of the Southeast Asia (16, 21). It is very rare in other populations. We have found, however, one $--^{FIL}$ mutation among our patients, and it was autochthonous child.

The improvement in molecular diagnostics allows to identify already known mutations and search for a new one. Among the most popular techniques are real-time PCR, gene sequencing, Southern blotting and ASO (allele-specific oligonucleotide probes) method used in our study (22). This technique is the best approach to examine populations with limited number of α globin

gene mutations. Although the frequency of -$\alpha^{3.7}$ mutation observed by us and other groups indicates that our population is similar to another already well studied ethnical groups, it is not excluded that some rare or novel mutations could be found in our patients, like in the case of β thalassemia (7). To resolve that issue, different approach is needed. The best solution would be elaborating and implementation of the method of sequencing of the whole α globin cluster, since the presence of α thalassemia may be connected with mutations not only in α globin genes, but also in regulatory element of the cluster located upstream of the α globin gene (9, 14). Sequencing of large sections of the genome is, however, difficult and laborious. Another approach could be working out of different indicator of α thalassemia, for instance some biochemical marker, connected with disturbed redox state, observed in various haemoglobinopathies and thalassemias (23-25).

CONCLUSIONS

Thalassemia α may become a growing health problem in parts of the world, where it was considered extremely rare. Therefore, appropriate diagnostic strategy is needed, where both well-known DNA-based techniques, and novel biochemical markers should be of great help.

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