Grażyna Krzemień, Agnieszka Turczyn, Agnieszka Szmigielska, Maria Roszkowska-Blaim

VIT. B_{12} DEFICIENCY IN CHILDREN (IMERSLUND-GRÄSBECK SYNDROME IN TWO PAIRS OF SIBLINGS)

NIEDOBÓR WIT. B_{12} U DZIECI (ZESPÓŁ IMERSLUND-GRÄSBECK U DWÓCH PAR RODZEŃSTWA)

Department of Pediatric and Nephrology, Medical University of Warsaw, Poland

Abstract

Improvement in the quality of life in Europe and North America in last decades caused that economical and social aspects of living conditions of the population have less effect and genetic defects of malabsorption of vitamin B_{12} became the main reason for cobalamin deficiency in children. Imerslund-Grasbeck syndrome (IGS) is characterized by vitamin B_{12} deficiency that leads usually to megaloblastic anemia and mild proteinuria. We described two pairs of siblings in two families with IGS. The diagnosis in first family (two brothers) was established at 33 and 22 months of age. The reason for diagnostic tests were proteinuria and anemia. Apart from respiratory tract infections, they didn't present other symptoms of cobalamin deficiency. In the second family IGS was diagnosed in children at 5 and 8 years of age. Diagnostic evaluation procedures were performed because of neurological signs, including weakness, loss of appetite, dysmorphia, psychomotor retardation. Laboratory tests revealed megaloblastic anemia, low concentration of vitamin B_{12} in serum and mild proteinuria. In the first pair low concentration of vitamin B_{12} was validated by the Schilling test, in the second pair methylomalonate acid was detected in the urinary metabolic test. All children were successfully treated with vitamin B_{12}, and anemia and neurological signs disappeared. Long-term follow up showed failure to thrive in the girl and physical and mental retardation, microcephaly in her brother. Proteinuria in the range: 0.3-1.2 g/24 h was detected in each child, and the other laboratory tests were normal. Clinical symptoms, laboratory tests and good reaction to parenteral treatment with vitamin B_{12} allowed us to diagnose Imerslund-Grasbeck syndrome, even without genetic tests.

Conclusion: *A delayed diagnosis of congenital malabsorption of cobalamin can lead to physical and mental retardation in children. Children with megaloblastic anemia and proteinuria resistant to classical treatment should be tested for congenital malabsorbtion of cobalamin.*

Key words: megaloblasic anaemia, vitamin B_{12} malabsorption, Imerslund-Gräsbeck syndrome, intristic factor deficiency, tubular proteinuria

Streszczenie

Poprawa jakości życia spowodowała, że genetycznie uwarunkowane zaburzenia wchłaniania witaminy B_{12} stały się główną przyczyną niedoboru kobalaminy u dzieci w Europie i Ameryce Północnej. Zespół Imerslund-Gräsbeck (IGS) charakteryzuje się niedoborem kobalaminy powodującym zwykle niedokrwistość megaloblastyczną i łagodny białkomocz. Opisano 2 pary rodzeństwa z IGS. U pierwszej pary (dwóch braci) rozpoznanie zespołu postawiono odpowiednio w wieku 33 i 22 miesięcy. Powodem badań diagnostycznych u chłopców był przypadkowo stwierdzony białkomocz i niedokrwistość. Poza infekcjami układu oddechowego, nie demonstrowali innych objawów niedoboru kobalaminy. U drugiej pary (chłopiec i dziewczynka) rozpoznanie postawiono odpowiednio w wieku 8 i 5 lat. Powodem badań u chłopca były zaburzenia neurologiczne, osłabienie, brak apetytu, dysmorfia, upośledzenie rozwoju fizycznego i zaburzenia rozwoju intelektualnego, u dziewczynki opóźnienie rozwoju fizycznego. W badaniach laboratoryjnych stwierdzono niedokrwistość megaloblastyczną, niskie stężenie witaminy B_{12} w surowicy i białkomocz. U pierwszej pary potwierdzono upośledzone wchłanianie witaminy B_{12} w teście

Schillinga, u drugiej wykazano obecność kwasu metylomalonowego w moczu. Po zastosowaniu leczenia parenteralnego witaminą B_{12} uzyskano normalizację parametrów morfologicznych i metabolicznych, ustąpienie zaburzeń neurologicznych. Długofalowa obserwacja wykazała u dziewczynki upośledzenie rozwoju fizycznego, u jej brata - upośledzenie rozwoju fizycznego, małogłowie i zaburzenia rozwoju intelektualnego, u wszystkich dzieci stwierdzano białkomocz w granicach 0,3-1,2 g/dobę, pozostałe badania laboratoryjne nie odbiegały od normy. Pomimo braku badań genetycznych, na podstawie objawów klinicznych, badań laboratoryjnych i odpowiedzi na leczenie parenteralne kobalaminą, można było z dużym prawdopodobieństwem rozpoznać u dzieci zespół Imerslund-Gräsbeck.

***Wnioski:** Opóźnione rozpoznanie wrodzonego zaburzenia wchłaniania kobalaminy może prowadzić do upośledzenia rozwoju fizycznego, małogłowia i zaburzenia rozwoju intelektualnego u dzieci. Dzieci z oporną na leczenie niedokrwistością megaloblastyczną powinny być diagnozowane w kierunku wrodzonych zaburzeń wchłaniania kobalaminy.*

Słowa kluczowe: niedokrwistość megaloblastyczna, zaburzenie wchłaniania witaminy B_{12}, zespół Imerslund-Gräsbeck, niedobór czynnika wewnętrznego, białkomocz cewkowy

DEV PERIOD MED. 2015;XIX,3,II:351-355

INTRODUCTION

Imerslund-Gräsbeck syndrome (IGS, (OMIM#261100) or juvenile selective malabsorbtion of vitamin B_{12} (cobalamin, Cbl) is a rare autosomal recessive disorder. It is characterised by vitamin B_{12} deficiency that leads to megaloblastic anemia and mild proteinuria. The cause of IGS is the defect of the receptor of the complex: cobalamin-gastric intrinsic factor (IF) of the erythrocytes in ileum. Biallelic mutations in CUBN gene (OMIM#602997) located on chromosome 10 or AMN gene (OMIM#605779) located on chromosome 14, encoding proteins: cubilin and amnionless respectively, are responsible for the disorder [1]. Both of the proteins cubulin and amnionless are subunits of the Cbl-IF receptor in ileum and receptor that takes part in the renal uptake of proteins from ultrafiltrate in proximal tubule. Receptors have probably identical or very similar conformation, but they differ from each other in the amount of ligands. The intestinal receptor has only one ligand: Cbl-IF, whereas renal receptor has at least a few and it mediates reabsorbtion of many proteins [2]. Cubulin – amnionless complex (cubam) is considered to be crucial not only for cobalamin absorption, renal uptake of proteins but also for early embryogenesis [3].

IGS was described for the first time in Finland and Norway in the sixties of the twentieth century. The frequency of IGS is unknow, but in Norway and Finland it is about 1: 200 000 [1]. The results of screening, which was established in Scandynavia revealed relationship between mutation on CUBN and AMN and occurence of IGS in this population. In different regions of the world (Turkey, Izrael, Saudi Arabia) high rate of consanguinity leads to transmission of the mutations that have already existed [3]. About 400 cases of IGS have been described in the world [1, 4].

CASE REPORT

First family

A boy with recurrent respiratory tract infections was hospitalized at the 19th months of age because of severe anemia, which has been ineffectively treated with iron and folic acid orally for 6 months. Blood tests revealed: HGB 4.8 g/dl, RBC 1.89 mln/ul, MCV 120 fl, MCH 36 pg, RET $44^0/_{00}$, PLT 26 x 10^3/ul, the iron concentration 44 ug/dl, the folic acid concentration in blood 3.1 ng/ml (n: 3.5-5.0 ng/ml), vitamin B_{12} level <10 pg/ml (n>200 pg/ml). The bone marrow biopsy was performed and it showed megaloblastic erythropoiesis. The first diagnosis was: anemia due to vitamin B_{12} deficiency caused by malabsorbtion of vitamin associated with antbioticotherapy. His urinalysis showed mild proteinuria 119-190 mg/dl. Schilling test was done at 33 months of age and it reveled malabsorbtion of vitamin B_{12} – 0,12% (n>10%). Parenteral treatment with vitamin B_{12} was prescribed. At the age of 15 years his physical growth, blood pressure, blood count and parameters of renal functions were normal, but proteinuria still persisted 0.7-1.2 g/24 h. His brother was accidentally diagnosed with anemia and proteinuria at the 21 months of age during hospitalization because of pneumonia. Blood tests revealed: HGB 6.5 g/dl, RBC 1.7 mln/ul, MCV 109 fl, MCH 35.2 pg, RET 5 ‰, the iron concentration 132 ug/dl, and urinalysis showed proteinuria 47-111 mg/dl. Schilling test was performed at the age of 22 months and malabsorbtion of vitamin B_{12} was diagnosed. Parenteral, life-long treatment with vitamin B_{12} was prescribed. When he was 10 years old his psychomotor development, blood pressure, blood count, renal parameters were normal, proteinuria was 0.4-0.7 g/24 h. The oldest brother, mother and the remaining members of the family on his father's side were healthy.

Second family

A boy was treated with iron and folic acid orally because of anemia in second and third year of life. He was hospitalized at the 8 years of age because of neurological symptoms (balance disorder, abnormal walking, intention tremor, excessive tendon reflexes), weakness, loss of appetite, dysmorphia, psychomotor retardation. Blood tests showed: HGB 6.7 gdl, RBC 1.73 mln/ul, MCV 104 fl, MCH 37 pg, RET 6‰, the iron

concentration 35 ug/dl, the vitamin B_{12} concentration 9 pg/ml, urinalysis mild proteinuria. The bone marrow aspiration revealed megaloblastic erythopoiesis with dominance of megaloblastic regeneration. The urinary metabolic test (GC-MS) revealed presence of methylomalonic acid in urine. After using chronic injections of vitamin B_{12}, neurological disorders disappeared, blood count became normal, and there were no methylomalonate in urine. Psychomotor impairment, microcephaly was detected at the age of 10. Blood pressure, blood count, renal parameters were then normal, but proteinuria was still present 0.38 g/24 h. His sister has been observed because of failure to thrive in the second year of life. Laboratory tests revealed: HGB 11.5 gdl, RBC 3.27 mln/ul, MCV 104 fl, MCH 35.1 pg, the vitamin B_{12} concentration: 35 pg/ml, proteinuria 215 mg/dl and the presence of methylomalonic acid in urine. She was treated with vitamin B_{12} given parenteraly. When she was 10 years old she was found to have psychomotor retardation. Her blood count, renal parameters were normal, and proteinuria was 0.3 g/24 h. Her two other siblings and parents were healthy.

DISSCUSION

Vitamin B_{12} is synthesized by bacteria in mammals digestive tract. Animal products are the source of the vitamin for humans. Under physiological conditions, the vitamin is released from eaten food, and then become bound to the glycoprotein-haptocorin (R-protein). In the small intestine it is liberated from the haptocorin, and then it becomes associated with IF, excreted by gastric mucous membrane. The complex Cbl-IF attaches to a receptor at the end of small intestine. It causes separation of Cbl which binds transcobalamin (TC). Cbl- TC complex is transported with blood into tissue and it is associated with specific receptors. Dysfunction at any stage of absorption of Cbl from ileum or during its circulation in blood leads to vitamin B_{12} deficiency [3, 4, 5]. Cbl is a cofactor for : methionine synthase which catalyzes the conversion of homocysteine to methionine in cytoplasm and mitochondrial methylmalonyl-CoA mutase which catalyses the conversion of methylmalonyl-CoA to succinyl-CoA. A deficiency of vitamin B_{12} results in elevated levels of methylmalonic acid and homocysteine, and decreased levels of methionine in body fluids [4, 6].

There are many reasons for cobalamin deficiency in childhood:

1) decreased dietary intake – vegetarian and vegan diet;

2) acquired malabsorption disorders: celiac disease, tropical sprue, diverticulitis, intestinal blind loops (overgrowth of bacteria which digest Cbl), parasitic infestation (fish tapeworm infection), lack of intrinsic factor (pernicious anemia due to: autoimmune atrophic gastritis and/or infection with *Helicobacter pylori*), errors during coenzyme synthesis (usage of laughing gas – nitrous oxide by nursing mothers);

3) congenital impairment of vitamin B_{12} absorption from gastrointestinal tract : defect of the receptor of the complex Cbl-IF, intrinsic factor deficiency, transcobalamin and haptocorin deficiency [1, 3, 4, 5, 7, 8, 9].

The quality of life improvements caused that genetic defects of malabsorbtion of Cbl - Imerslund-Grasbeck syndrome and inherited insufficiency of IF became a main reason of Cbl deficiency among children in Europe and North America [1, 9]. The reason of congenital IF deficiency (IFD; OMIM#261000) is recessive mutation in gene GIF (OMIM#609342) located on chromosome 11, coding for the synthesis of IF [1].

The inherited deficit of IF appears less often than IGS. Tanner during his genetic studies among 154 families with hereditary defect of malabsorbtion Cbl evaluated prevelance of mutation in genes: CUBN, AMN i GIF as 42%, 36% and 22% retrospectivelly [10]. More than 100 cases of IFD have been described in the world until now (5).

The clinical picture and laboratory abnormalities don't differ in IGS and IFD [1, 11]. A congenital malabsorption Cbl has been recognized most often from the age of several months to several years, rarely in adults [1, 11, 12, 13]. A low concentration of Cbl in pregnant woman (vegan/vegetarian diet) can be the reason of brain and neural tube malformations in fetus, megaloblastic anemia and neurological disorders in a newborn. The screening of IBS – gas chromatography mass spectrometry (GC-MS) of urine allows for early detection of Cbl deficiency [4, 7]. Patients with Cbl insufficiency usually present nonspecific clinical symptoms: physical impairment, psychomotor regression, difficulties in feeding, paleness, fatigue, recurrent respiratory and gastrointestinal tract infections, visual disorders (macular degeneration). Neurological and psychiatric symptoms appear rarely and are mild. Clinical sings are connected with accumulation of methylomalonate and homocystein in body fluids [1, 3, 6, 7, 12].

The IGS assessment for the first pair of siblings was established at 33 and 22 months of age. The reason why diagnostic tests were performed on both boys was proteinuria and anemia. Apart from recurrent respiratory tract infections, they didn't present other symptoms of cobalamin deficiency. The anemia due to vitamin B_{12} and folic acid deficiency as the result of malabsorbtion disorders after taking antibiotics, was incorrectly diagnosed when the boy reached 19 months. In the second pair evaluation was made at 5 and 8 years of age. The boy had laboratory tests because of: neurological disorders, weakness, loss of appetite, dysmorphia, psychomotor retardation, and the girl was diagnosed due to of physical retardation.

The cobalamin deficiency can manifest only in laboratory tests. The reason why diagnostic tests for IGS and IFD are done in children is usually: megaloblastic anemia and/ or proteinuria, rare thrombocytopenia, neutropenia or pancytopenia [1, 5, 7, 11, 12]. The major reason of the illness is reduction of erythropoesis due to impaired maturation of erythrocyte progenitor. The lack of megaloblastic anemia among some people with Cbl deficiency, may results from taking large amounts of folic acid (vegetarian diet). It may mask anemia, which is caused by Cbl deficiency and increases the neurological disorders [1, 3].

The proteinuria is recognized in about 50% of patients with IGS [14] and only occasionally among patients with

IFD [4]. In IGS floating proteinuria is persistent without other symptoms of renal disease. The analysis of urinary proteins excretion from patients with IGS showed a presence of albumin, transferrin, light chains of immunoglobulin, α_1 and β_2-microglobulins, A1-apolipoprotein, vitamin D binding protein (DBP), myoglobin and hemoglobin [1, 2, 13, 15, 16]. Storm described correlation between the type of mutation discovered in 9 patients with IGS and the kind of low-molecular- weight proteinuria [16]. The cause of proteinuria in IFD is unknown. The presence of mild asymptomatic proteinuria strongly suggests IGS [3, 12, 13]. Renal biopsy performed in untreated patients with IGS was generally normal or revealed only very slight changes on light and electron microscopy – slightly increased thickness of glomerular basement membrane, proliferation of mesangial cells, presence of mesangial deposits. Biopsy after treatment with vitamin B_{12} didn't show any changes. However, single samples from renal biopsy, which was taken from patients with Cbl deficiency showed significant aberrations: for example MPGN with features of thrombotic microangiopathy [6].

To establish the causes of megaloblastic anemia, the concentration of folic acid and vitamin B_{12} should be checked. A therapeutic test with Cbl can be helpful with differential diagnosis. The rise in the concentrates of blood cells and hemoglobin after parenteral injections of vitamin B_{12} allows diagnosis of Cbl deficiency. Detailed tests should be performed after supplementation of the Cbl deficiency [1]. However, differentiation between IGS and IFD can be difficult. The Schilling test was done in the past and it was used to evaluate the ability to absorb vitamin from the digestive system. This test is based on the measurement of the radioactivity of cobalt-labeled cobalamin excreted in urine. In patients with IFD, oral administration of radioactive Cbl with IF caused increase in absorption of cobalamin (increase in the Schilling test). In patients with IGS low absorption of Cbl is not corrected with IF supply. However, the Schilling test was withrawn because of lack of availability of the radioactive-Cbl, and its high costs and relatively complicated test procedure [1, 3, 5]. The absorption test (holo-TC) with the use of non-radioactive cynanocobalamin (CN-Cbl), that bounds with transcobalamin in the blood can replace the Schilling test. The measurement of TC associated with CN-Cbl allows evaluation of the absorption of vitamin B_{12} [1, 7].

In real clinical setting the prevaleance of IGS can be lower than it was previously expected. A simple Schilling test and a test corrected with administration of IF haven't been always available. Because of uncertain diagnosis, some cases of IFD could have been incorrectly recognized as IGS. It particularly applies to patients without detectableproteinuria [16]. Tanner performed genetic research for IGD in some families, which were initially without genetic tests positive for IGS [9]. The final diagnosis of IGS or IFD can be established if biallelic mutation in gene CUBN or AMN, coding for subunits of the Cbl-IF receptor or GIF gene coding IF have been confirmed. Unfortunatelly, genetic tests are relatively complicated, because of the large amount of gene polymorphism and because they are expensive. In fact, the low reported frequency of occurrence of IGS and IFD is most likely caused by the lack of cheap, commercially available genetic tests [1].

The laboratory tests in the studied children revealed megaloblastic anemia, low concentration of vitamin B_{12} in serum and proteinuria. In the first pair of siblings, a low concentration of vitamin B_{12} was validated by the Schilling test (the test corrected by application of IF wasn't available). In the second pair methylomalonate was detected in urine. After parenteral treatment with vitamin B_{12}, normalization of blood counts, metabolic parameters and neurological disorders were achieved. Long-term observation showed physical retardation in a girl and physical and mental retardation, microcephaly in her brother. Proteinuria (0.3-1.2 g/24 h) was detected in each child. Other laboratory tests were normal. In spite of the absence of genetic tests, taking into account clinical symptoms, laboratory tests and response to parenteral treatment with cobalamin, Imerslund-Grasbeck syndrome can be recognized with high probability.

The treatment of IGS and IFD consists of chronic parenteral lifelong injection of vitamin B_{12}. Cobalamin is administered once a month. Abdallah described 4 patients with IGS and 3 with IFD in whom administration of the vitamin B_{12} twice a year was enough to control normal blood count and metabolic parameters [12]. Proteinuria is not reduced after treatment with Cbl, probably because of the invalid function of renal receptor. Some patients observed for over 50 years had no reduction of renal function [1, 13]. If sufficient dose of vitamin B_{12} is administrated the prognosis in IGF and IFD can be good [1].

CONCLUSION

Delayed recognition of congenital malabsorption of cobalamin can lead to physical and mental retardation in children. Children with megaloblastic anemia resistant to classical treatment should be evaluated for congenital malabsorption of cobalamin.

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Author's contributions/Wkład Autorów
According to the order of the Authorship/Według kolejności

Conflicts of interest/Konflikt interesu
The Authors declare no conflict of interest.
Autorzy pracy nie zgłaszają konfliktu interesów.

Received/Nadesłano: 12.05.2015 r.
Accepted/Zaakceptowano: 26.05.2015 r.

Published online/Dostępne online

Address for correspondence:
Grażyna Krzemień
Department of Pediatric and Nephrology,
Medical University of Warsaw, Poland
Marszałkowska 24, 00-576 Warsaw, Poland
tel. (+48-22) 52-27-388
fax. (+48-22) 621-98-63
e-mail: grazyna.krzemien@litewska.edu.pl