UDK 611(082) ISSN 1512-8245

AKADEMIJA NAUKA I UMJETNOSTI BOSNE I HERCEGOVINE
АКАДЕМИЈА НАУКА И УМЈЕТНОСТИ БОСНЕ И ХЕРЦЕГОВИНЕ
ACADEMY OF SCIENCES AND ARTS OF BOSNIA AND HERZEGOVINA

RADOVI

KNJIGA XCII

Odjeljenje medicinskih nauka
Knjiga 31
Centar za medicinska istraživanja
Knjiga 2

SARAJEVO 2003

GENETIICAL MUTATION OF HROMOSOME 1 - MORBUS GAUCHER TYPE I

- Case report -

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Introduction

Modern diagnostics, with aid of advanced instrumental technics, almost ever day discovers new forms of pathological conditions in human population caused by various genetical factors.

Up to date has been registered more than 3 600 various forms of diseases determined by various forms of gentical disorder in genotype (McKusick, 1982). Their distribution and frequency is various, and it depends on both endogenous and egzogenous factors, and even on belonging to the certain ethnical groups (Kingama et al. 1998).

Investigations of the frequency of congenital malformations is today one of the high priority tasks in Bosnia and Herzegovina. This enables development of successful prevention, as well as finding of adequate measures of modern therapy on the basis of achievements of gene therapy.

One od such diseases is a very rare genopaty Morbu Gaucher type 1 which will be presented in this paper in more details.

What is Morbus Gaucher type 1?

Morbus Gaucher is lisosome disease of lypide deposition. It was described by French dermatologist Ph. Gaucher in his dissertation from 1882. Its family character and more detailed description was given by German scientist F. Schlagenhaufer in 1907. Defect of enzyme betaglycozydase (glycocerebosidase), which play a role in decomposition of

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cerebroside lypides and it is located in autosome 1, is caused by this disease.

This leads to the accumulation of parts of not completely decomposed cerebrosides, which are particularly numerous in reticule-endotel cells of spleen and bone marrow - Gaucher cells. The disease is relatively benign, and ita has gradual progression and it last for decades. Particular attention is paid to the therapy, that is to the supstitution of adequate enzyme, which is very expensive. (Grabowski, 1998).

It should be noted that never in the same family was described more than one type of Gaucher disease, although this disease is inherited via autosomal-recesive way.

There are three forms of this disease, and all of thema are the result of mutation on hromosome 1. This leads to decrease of activity of glucocerebrosidase. This enzyme cuts glucose from glucocerebroside (glycozylceremide) composed of equimolar amounts of amino alcohols of long chains of sphingosine, fatty acid in long chain, and glucose. Described lypide is accumulated mainly in cells of reticular endotels, which become alike Gaucher cells size frm 20 to 100 microns. They could be easily found in red pulp of spleen, in bone marrow and in sinusoides of liver (Grabowski, 1998).

Type 1 is a chronic or adult relatively benign form without consequences on CNS, which could give syptoms from school children age or later on. It is manifested through increase of spleen size as the result of increase of level of glucosil-ceremides in cells of RES. The next stage is hepatomegaly, and pains in bones and joints begin due to the infiltration of bone marrow with pathological fractures and aseptic osteonecrosis. The disease lasts for decades, but possible complications in osteoarticular system make prognostics more difficult.

Acute malign form, type II, begins between one and six months of age with apathy, strabism, opistotonuse, and attacks of laringospasm. Sometime appears convulsions. A large number of children dies at age of nine months, and main cause od death is anorexy and lungs infection.

Juvenile, subacute, neuronopathical form of the disease (type III) represents transition between ttype I and type II of the disase. Only a few cases of this form of the disease was described. A lrage number of children has convulsions with abnormal EEG. It begins after age of three years and neurological symptomes have slow progress in comparison to the acute form, and for these reasons patients could reach adult age.

Defintive diagnosis could be obtained by proving of decrease or lack of activity of enzyme in leucocites in blood or fibroblast of skin. Enzyme test is the only one specific method for diagnostics. In addition, DNA analysis could be carried out. Prenatale diagnostics of the disease could be determined by cultivation of cells of amnion liquor and by the

determination of activity of beta-glycosidase in them There is also apparent increase of acid phosphatase activity. Investigation of activity of that enzyme in leucocites and fibroblasts could help in determination of heterozygote vectors of this disase (Pastores, 1998).

Materials and methods

Gaucher morbus type 1 was determined in girl (age 14) in Cantonal hospital «Dr. Irfan Ljubijankić" in Bihać (Fig. 1). The clinical investigations were carried out at the same hospital, and samples necessary for the further detailed biochemical analysis were taken together with all other relevant data for this disease.

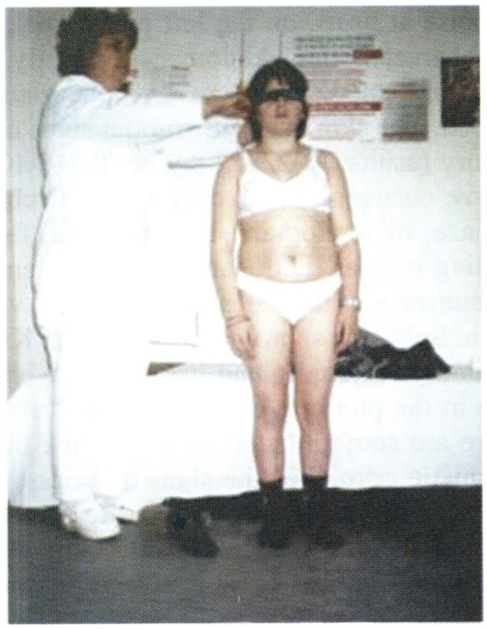

Fig. 1. *Patient with Gaucher morbus type 1*

A contact with the Institute for metabolic disorders in Austria was i made in order to obtain a definitive diagnostics. It was necessary to determine enzymes in skin fibroblast. Biopsy was made and material was sent for analysis to professor Paschke, Lab. for metab. Disorders UNI Graz.

All available genealogical methods were used in order to determine «genetical identity» and source and form of transfer of Gaucher gene.

Results and discussion

Case description

A girl (age 14) came to the Department of pediatrics clinic of the Cantonal hospital "Dr. Irfan Ljubijankić" due to the abundant and prolonged menstrual bleeding, increased liver, spleen and anemia (Fig.1). The girl was born as seventh child in healthz mother. Perinatal and postnatal period were regular. Up to this period the girl was not ill. The first menstruation appeared at age of 13.5. The second menstraul bleeding came after five months and it lasted for six days. After the break that lasted for two days bleeding started again with short breaks until coming to the hospital.

In status: afebrile, pale, lower height at brests, finding is regular. There is a noise at heart, on the left parasternal, of intensity II/VI, with functional characteristics. Abdomen is slightly distended, liver is palped for 2 cm, and spleen for 7 cm. Lymphe glands on predilection sites are not palped. The skin is clean, without signs of bleeding.

Laboratory results: E: 2.9 L: 8.9 hct 0.21 MCV 72 Tromb 265. DBP without any changes. Urea, creatinin, bilirubin, urin, proteinogram, transaminaze, mineralogram, koagulogram, finding regular. Gynecological finding regular. Ultrasound of abdomen showed increases liver with pronounced intrahepatical portal blood vessels. Portal vein has limited width. Spleen is significantly enlarged. Kidney ultrasound is regular. Ultrasound of thyroid gland has shown a bit decreased size of the gland. Since in the punctate of bone marrow and spleen exist large foamy cells there are some indices on Niem-Pick disease. After transfusion of depalsmatic eritrocites the signs of hemoragical dietesis have stoped, and the patient was sent home. She receives iron peroraly and vitamine C. Blood picture and trombocites are controlled once per month. She ocassionaly suffers pains in spleen region. Menstraul cycles are regular.

Table 1. *Values of analysed enzymes*

Enzyme	Patient	Control	Normbereich	Nachweis von
β-Glucosidase	0	1.41	1.5-4.7	M.Gaucher
Sphingomyelinasc	7.56	9.99	9.4-15.6	M.Niemann-PickA/B
β-Galaktosidasc	8.04	4.32	3.2-24.0	GMI-Gangliosidose MPS IVB
β-Hexos. Thermostabilitata (%)	42	53		M.Tay-Sachs
β-Hcxos., 4-MU-GlcNAc-6-S	5.79	6.38		M.Tay-Sachs

Values of enzymes in cultivated skin fibrobalsts are presented in Table 1. Activity of beta-glycosidase is not measurable. This is the evidence of Gucher disease. Value of acid phosphatase was high.

There is no significant difference between M.Gaucher and M. Nieman-pick.

Genetical aspects

Gaucher gene is type of proteine and this gene is defective and it is not capable to fulfill its normal function.

The patient was born as the seventh child in the family. Parents, brothers and sisters are healthy, except for one child which died at age of three months due to suffocation. One child in the family of uncle has epilepsy and it is psychically retarded.

"Gaucher gen" is located on autosome number 1. Patient with Gaucher disease must inherit two deffective copies of the gene (one from both parents). If both parents have normal genes, and one of them is carrier of the gene for Gaucher disease while and other is not, there are 50% chances that child will inherit gene and become disease carrier ("Geucher gena"). Children of parents carriers will not have Gaucher disase since the children will have normal gene collection from other parent.

If both parents are carriers of Gaucher gene there are 25% with each pregnancy that children will inherit that gene and in that way they will become disease carriers.

In this case, taking into acount rules of distribution of certain genes responsible for transfer of characteristics and processes, and following genealogical series, it could be concluded that parents of the patient are heterozygotes (healthy) and carriers of Gaucher gene, and that ¼ of their children are dominant homoyzgotes (healthy), 2/4 are heterozygotes (healthy) and carriers of Gaucher gene, and ¼ of children, together with the patient, are recessive homozygotes, and carriers of Gaucher gene with expression of the type 1 of this disease. If both parents are carriers of Gaucher disease there is 25% of probbility in each pregnancy that child that inherited that gene would become disease carrier.

Disscusion

Clinical situation

The most pronounced is cerebral syptomatology: disturbance, high sensitivity, brain spasms, opistotonus, stabism. The resistance to ifections is decreased. All these changes decrease working capabilities

and make problems. Prenatal diagnostics is possible (Kičić&Krajinčanić, 1989).

Constant attention of hematologist is necessary due to the trombocytopenia. It would be necessary to pay attention to the development of anemia. Splenectomy could yield good results when there are signs of hyperspalnism. However, this operation should be postponed as long as it is possible, since after splenectomy begins increased production of Gaucher cells in liver and bone marrow which accelerate insufficience of these organs. The only adequate therapy is supstantion of enzymes which is in wide use today. This therapy is very expensive and it is implemented in the form of injections of purified glucocerebrosidase obtained from human placente (ceredasa, and modified form of glucocerebrosidase - Ceresyme, which use is decreased price of therapy and there are no evidence of increase of antibodies in relation to aglucerase (Table 2).

Table 2. *Therapeutical results of use of certain drugs on Gaucher disease*

	Ceredase	**Cerezyme**
U.S. Generic: Name	*Aglucerase injection*	*Imiglucerase for injection*
Molecule	*Human placental protein (endogenous); trace human chorionic gonadotropin*	*Recombinant; one amino acid change (R495H)*
Vial size (units)	*400;50*	*200*
Formulation	*Solubilized*	*Lyopphilized*
Additives	*Citrate Human serum albumin (1%)*	*Citrate Mannitol Polysorbate 80*

The therapy with this enzyme was for the first time carried out in 1989, and since 1991 it is in wide use. Therapeutical dose is 60U/kg i.v. every other week. There were certain attempts to implement twice smaller dose, but there were no expected results. More than 2000 patients from all over the worls was treated with this enzyme. The study carried out by Gregory A. Grabowski in Human Childrens Hospital, Cincinnati, Ohio, USA involved 17 female and 13 male patients which have been parallely treated with aglucerase and imiglucesae. The study included measurements of values of acid phosphatase, bilirubin, hemoglobyne, trombocytes, leucocytes, iron, volumen of liver and spleen estimated with CT and MRI. Antibodies maesurements were also carried out. Evaluation included period of nine months. The dose was 60 U/kg every second week (Grabowski, 1998).

Increase of values for hemoglobine and number of trombocites after sic months and after nine months of therapy is presented in Table3. There are no significant differences in values between therapy with ag-

lucerose and imiglucerose. The volume of liver and spleen decreased for 34.7% after six months of therapy. Acid phosphatase decreased for more than 30%. Antibodies at beta-glycosidase was present in six patients treated with aglucerase and in three patients treated with imiglucerase.

Table 3 - *A comparative presentation of the results of the therapy with aglucerase and imiglucerase (Grabowski, 1998)*

Enzyme	Hemoglobin			Platelets		
	Initial (Δg/dL)	6 mo (Δg/dL)	9 mo (Δg/dL)	Initial (x10³/mm³)	6 mo (% Δ)	9 mo (% Δ)
Aglucerase	10.77 (8.7 to 12.8)	↑1.60 (-0.35 to 3.1) 13/15>1.0 9/15>1.5	↑2.28 (0.5 to 4.25) 13/15>1.5	70.9 (28 to 138)	19.600 (↑33.5%) (1.6 to 123%) 7/15 > 20%↑	30.100 (↑53.2%) (-23 to 210%) 7/15 > 40%↑
Imiglucerase	10.71 (6.0 to 13.6)	↑1.82 (0 to 4.3) 12/15 > 1.0 8/15>1.5	↑2.54 (0.4 to 5.8) 10/15>1.5	72.1 (28.5 to 133.5)	16.100 (↑21.5%) (-21 to 87.5%) 7/15>20%↑	28.773 (↑43.5%) (-6.3 to 95%) 7/15 > 40%

Possible therapy in our patient

Cells obtained from punctate of bone marrow or spleen are similar, but there is an difference regarding therapy. Gaucher disease is the first lysosome disease where enzymatical therpay was implemented. There are enzymes in the market, but treatment is very expensive. Annual costs of the treatment for our patient would be some 250000 euros. There are no registered other cases of this disease in other pediatrics clinics in Bosnia and Heryegovina, and in neighbouring Croatia. Three cases are registered in Austria, where all costs of treatments are coverd by the state. Of course, we do not have such a possibility, and therefore the girl could rely only on symptomatical therapy. Since Gacuher disase type 1 has slowly progress, the prognosis is not so bad. It is not possible to determine when the first signs of hypersplenism, bone marrow changes and other complication will take place.

Genetical aspects

Having one child with Gaucher disase does not mean that next three children will inherit this disease. If one of the parents has this disease or he/she is the carrier of the disease, all children will get the gene for Gaucher disease, and they could become ill, or they could become carriers of gaucher gene inherited from the parents. Therefore, gaucher disease is a form of "Genetical disorder" and there is a Gaucher gene in the family.

Families with anamnesis of Gaucher disease should participate in discusion on this disease with the doctor. It would be necessary to take a blood sample for the analysis of glucocerebrosidase in order to determine carrier. Blood tests are not always suitable due to the variations at enzyme level, level of acid phosphatasis, and glucocereb-rosidase.

Amnioentesis and causes of chorion samples could be used for determination of Gacuher disease in earlz stage of pregnancy.

Genetic consulting is possible for pairs that are carriers or that have in family cases with Gaucher disease (Rice et Barranger, 1996).

Conclusion

Maucher gaucher is a rare disease. It occures once in 40000 to 65000 persons in the whole world. This is the most frequent lysosome disease in the clinics. This is the first lysosome disease used in perinatal diagnostics and the first one where enzyme therapy was implemented.

Case of gaucher disease morbus type 1 was found in girl of age 14 in Cantonal hospital «Dr. Irfan Ljubijankić" in Bihać. The clinical investigations were carried out at the same hospital, and samples necessary for the further detailed biochemical analysis were taken together with all other relevant data for this disease.

In this case, taking into acount rules of distribution of certain genes responsible for transfer of characteristics and processes, and following genealogical series, it could be concluded that parents of our patient are heterozygotes (healthy) and carriers of Gaucher gene, and that ¼ of their children are dominant homoyzgotes (healthy), 2/4 are heterozygotes (healthy) and carriers of Gaucher gene, and ¼ of children, together with the patient, are recessive homozygotes, and carriers of Gaucher gene with expression of the type 1 of this disease.

For therapy of Gaucher disease there are in the market two enzyme. Aglucerosis obtained from human placente and imiglucerasis obtained from cells of ovaria of hamster. The second preparation is cheaper and it is in wide use. There are no differences in the activity, as well as in creation of antibodies - the enzyme entered in use for the first time in 1989, and since 1991 is in wide use. In Bosnia and Herzegovina, therapy is not possible due to the financial restrictions.

Apstrakt

Maucher gaucher je rijetka bolest. Javlja se jedan slučaj na 40000 do 65000 osoba cijelog svijeta. To je njačešća lizozomalna bolest u klinikama cijelog svijeta. To je prva lizozomalna bolest koja se koristi u perinatalnoj dijagnostici i prva kod koje je primijenjena enzimatska terapija.

Slučaj Gaucher morbus tip 1 je utvrđen kod djevojčice stare 14 godina u Kantonalnoj bolnici «Dr. Irfan Ljubijankić" u Bihaću. U istoj bolnici djevojčica je klinički ispitana, uzeti su neophodni uzorci za detaljnije biohemijske analize kao i drugi relevantni podaci za ovu bolest.

U ispitivanom slučaju, uz uvažavanje zakonitosti distribucije pojedinih gena odgovornih za transfer osobina i procesa, te genealoškim slijedom, može se konstatirati da su roditelji naše pacijentice heterozigoti (zdravi) i nosioci Gaucherovog gena, da je ¼ njihove djece dominantni homozigoti zdravi, 2/4 heterozigoti zdravi i nosioci Gaucherovog gena, a ¼ djece u koju spada i ovo dijete, su recesivni homozigoti, nosilac Gaucherovog gena kod kojeg je došlo do ekspresije ove bolesti tipa 1.

Za terapiju Gaucherove bolesti, na tržištu postoje dva preparata enzima. Agluceraza koja se dobiva iz humane placente i imigluceraza koja se dobiva iz ćelija ovarija hrčka. Ovaj drugi preparat je jeftiniji, te je naišao na široku primjenu. Nema razlike u načinu djelovanja, kao ni u stvaranju antitjela- enzim je prvi put naišao na primjenu 1989 godine, a od 1991 godine je u širokoj upotrebi. Kod nas iz finanasijskih razloga ta terapija je još neprihvatljiva.

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