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CHALLENGES IN CLINICAL CONSIDERATIONS FOR CONGENITAL DISORDERS OF GLYCOSYLATION

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REZUMAT

PROVOCĂRI ÎN CONSIDERAȚIUNI CLINICE PRIVIND DIAGNOSTICUL DEREGLĂRILOR CONGENITALE ALE GLICOZILĂRII

Tulburările congenitale ale glicozilării (CDG) reprezintă un grup de patologii monogenice determinate de defecte genetice ce perturbă procesele de glicozilare cu importanță primordială în biosinteza glicoproteinelor și glicoconjugatelor. Simptomatologia CDG sunt ultrarare, prevalența acestora fiind cuprinsă între 0.1-0.5/100.000 locuitori 70% corespunzând tipului CDG Ia (PMM2-CDG), cu o frecvență de 1:20.000 locuitori. Majoritatea tipurilor de CDG se prezintă prin afectări multisistemice (80% implică afecări neuologice, 22% - hepatice, 20% - cardiace, 20% - dermatologice, 10% - immunologice, etc.), determinându-se o heterogenitate de simptome clinice deseori cu caracter invalidizant. Variabilitatea manifestărilor mimează alte patologii ceea ce reprezintă o provocare pentru clinicieni, deseori CDG fiind subdiagnosticată. Metoda de elecție pentru diagnosticul CDG este Focusarea Izoelectrică a Transferinei [IEFT], propusă ca instrument de screening încă din 1984 de către Jaeken. Un număr de 40 de pacienți moldoveni suspecți pentru CDG au fost trecuți prin screeningul IEFT în colaborare cu Laboratorul de Translare Metabolică Radboudumc, Nijmegen, Olanda și S.U.A. Manifestările clinice depistate la pacienții incluși în studiu au fost foarte variate. În urma screeningului prin IEFT s-a determinat: 37 profiluri normale și 3 anormale cu suspiciuni pentru CDG. Prezența Fructozemiei și a Galactozemiei, care induc tulburări secundare ale glicozilării au relevat un profil anormal al trasferinei, de aceea este necesară excluderea acestora prin teste biochimice și secvențierea AND-ului. Varietatea manifestărilor clinice prezintă o provocare pentru diagnosticul CDG și chiar subdiagnosticarea acestuia.

Cuvinte-cheie: dereglări congenitale ale glicozilării, manifestări clinice, afectare multisistemică, maladie rară.

РЕЗЮМЕ

НАРУШЕНИЕ ГЛИКОЗИЛИРОВАНИЯ: ОТ МНОГООБРАЗИЯ КЛИНИЧЕСКИХ ПРОЯВЛЕНИЙ К ДИАГНОЗУ

Врожденные нарушения гликозилирования (CDG) представляют собой группу моногенных патологий, вызванных генетическими дефектами, которые нарушают процесс гликозилирования, имеющий первостепенное значение в биосинтезе гликопротеинов и гликоконъюгатов. Большинство типов CDG оченъ редкие, их распространенность составляет от 0.1 до 0.5 на 100.000 населения, 70% соответствует CDG Ia типа (PMM2-CDG), с частотой 1: 20.000 населения. CDG проявляются мультисистемными расстройствами: неврологическими (80%), печеночными (22%), сердечными (20%), дерматологическими (20%), имунологическими (10%) и др.), что приводит к гетерогенности клинических симптомов. Выборочным методом для диагностики CDG является изоэлектрическое фокусирование трансферина (IEFT). 40 молдавских пациентов с подозрением на CDG

были обследованы через IEFT в сотрудничестве с Лабораторией метаболической трансляции RadboudUMC, Неймеген, Нидерланды и США. Клинические проявления у пациентов, включенных в исследование, были разнообразными. После скрининга IEFT в 37 случаях определен нормальный профиль трансферина, а в 3 случаях – подозрение на CDG. Двоим из них были выставлены диагнозы фруктоземия и галактоземия. Эти патологии являются вторичными аномалиями гликозилирования и также характеризуются аномальным профилем трансферина. Клинический полиморфизм затрудняет диагностику CDG.

Ключевые слова: врожденные нарушения гликозилирования, клинические проявления, мультисистемное вовлечение, редкое заболевание

Introduction

Congenital disorders of glycosylation (CDGs) represent a group of monogenic pathologies caused by genetic defects in various steps in the biosynthesis of glycoproteins and glycoconjugates in ER/GA. The group of these pathologies are divided into disorders of N-glycosylation, O-glycosylation, mixed (N-and O-glycosylation), glycosphingolipid and glycosylphosphatidylinositol (GPI-anchor) synthesis. The most of these monogenic diseases are autosomal recessive in inheritance, but autosomal dominant and X-linked forms have also been described. The incidence and prevalence of all types of CDG have not been well established, although patients have been reported worldwide from almost every ethnic background and both sexes are equally affected. The estimated prevalence in European and African American populations is 1/10,000 based on carrier frequencies of known pathogenic variants in 53 genes. The prevalence of the most commonly diagnosed CDG, PMM2-CDG, ranges from 1/20,000 in Dutch populations and 1/77,000 in Estonia based on isolated reports [1]. According to the literature, there are reported over 150 CDG types

Table 1. Clinical features suggestive for CDG

Clinical features	Suspected CDG	Clinical features	Suspected CDG	Clinical features	Suspected CDG
Achalasia	GMPPA-CDG	Alacrima	GMPPA-CDG	Joint laxity	XYLT1-CDG
Anemia (dyserythropoietic)	SEC23B-CDG	Anorectal malformation	PIGV-CDG	Obesity	MAN1B1-CDG
Autonomic dysfunction	GMPPA-CDG	Behaviour disturbances	ALG6-CDG	Exostoses	EXT1-CDG EXT2-CDG
Bombay blood group	SLC35C1-CDG	Diaphragmatic hernia	PIGN-CDG	Myopathy	GNE-CDG
Calcinosis	GALNT3-CDG	Cardiomyopathy	ALG1-CDG DOLK-CDG DPM1-CDG DPM3-CDG PMM2-CDG	Liver fibrosis	CCDC115-CDG MPI-CDG PMM2-CDG TMEM199-CDG
Cataract	SRD5A3-CDG XYLT2-CDG	Coloboma	PIGL-CDG SRD5A3-CDG	Diarrhea (chronic)	ALG6-CDG ALG8-CDG MPI-CDG
Cerebrocostomandibular syndrom	COG1-CDG	Cutis aplasia	EOGT-CDG	Eye abnormality	B3GALTL-CDG
Cutis laxa	ATP6AP1-CDG ATP6V0A2-CDG ATP6V1A-CDG ATP6V1E1-CDG COG7-CDG GORAB-CDG	Deafness sensorineural	ALG11-CDG CHSY1-CDG PIGL-CDG RFT1-CDG XYLT2-CDG	Brahidactily	ALG6-CDG CHSY1-CDG PIGV-CDG
Inverted nipples	PMM2-CDG	Limbs defects	EOGT-CDG	Uvula/palate cleft	PGM1-CDG
Dwarfism (adult)	FUT8-CDG NANS-CDG TMEM165-CDG	Myastenia (congenital)	ALG2-CDG ALG14-CDG DPAGT1-CDG GFPT1-CDG	Ichthyosis	DOLK-CDG MPDU1-CDG PIGL-CDG SRD5A3-CDG
Retinitis pigmentosa	DHDDS-CDG	Hiperthermia (episodic)	COG7-CDG	Radio-ulnar synostosis	MGAT2-CDG
Hyper-/hypopigmentation	POFUT1-CDG POGLUT1-CDG	Fat pads	PMM2-CDG	Imune deficiency	MOGS-CDG PGM3-CDG SLC35C1-CDG

and 1350 patients diagnosed, with a distribution of 94% cases of CDG type I and 6% respectively CDG type II. With the development of metabolomics and glycomics, the number of CDG forms has increased exponentially, so that every 17 days a new form of CDG was confirmed in 2013, and in the first half of 2017, 5 new forms of CDG were detected [2 ,3]. Considering that at least 2% of the human genome encodes proteins involved in glycan biosynthesis and their recognition, and 5-10% of proteins are involved in homeostasis of Golgi apparatus with an effect on glycan metabolism, in the near future, will be reported new types of CDG [3].

For the first time, this group of pathologies was reported in 1980 by Jaeken, who in 2011 described CDG as "nearly the whole medicine in a nutshell" referring to its clinical heterogeneity (tab.1), which represents a real challenge for clinicians [4]. Most types of CDG are multisystem disorders, being involved almost all systems and organs, reflected by a variety of clinical symptoms (80% neurological manifestations, 22% - hepatic, 20% - cardiac, 20% - dermatological, 10%- immunological, etc.) and mimicking other pathologies which determines the underdiagnosis of CDG [5, 6,7, 8, 9]. The CDG diagnostic process is a complex one and includes biochemical screening methods to identify the glycosylation defect and analytical methods (mass spectrometry, whole exome/genome sequencing) to determine the causes and type of CDG [10,11,12]. The "gold standard" for CDG diagnosis is screening by isoelectric focusing of transferrin (IEFT). Following the IEFT analysis, two large groups of CDGs - CDGs type I and II can be established. The abnormal isoelectric profile of transferrin may also be caused by secondary factors such as fructosemia, galactosemia, genetic polymorphism of transferrin, alcoholism, severe hepatic impairment, which mimics CDG at the biochemical level. The latter requires the use of specific methodologies to eliminate the influence of secondary factors on the final diagnosis [12].

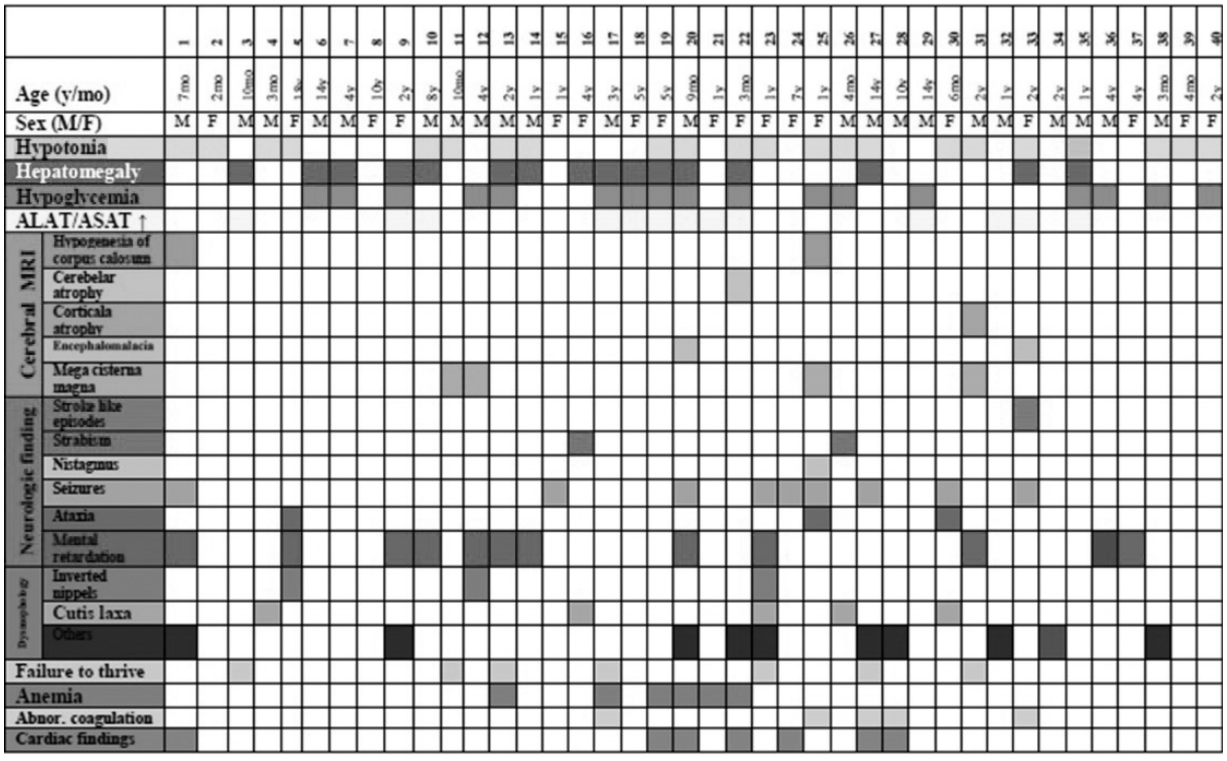
Material and methods

By medical-genetic consultation in Genetic Department of Institute of Mother and Child from Chisinau, there were selected 40 Moldavian patients of various ages (2mo–15y) clinically suspected for CDG. The basic metabolic investigation was performed in all patients for differential diagnosis. The analisys of organic acids in urine was performed by ^{1}H NMR spectroscopy at the "Petru Poni" Institute of Macromolecular Chemistry of Romanian Academy, Iasi, Romania. Screening for CDG by IEFT was performed for all our patients in collaboration with Translational Metabolic Laboratory Radboudumc, Nijmegen, Netherlands and U.S.A.

Results

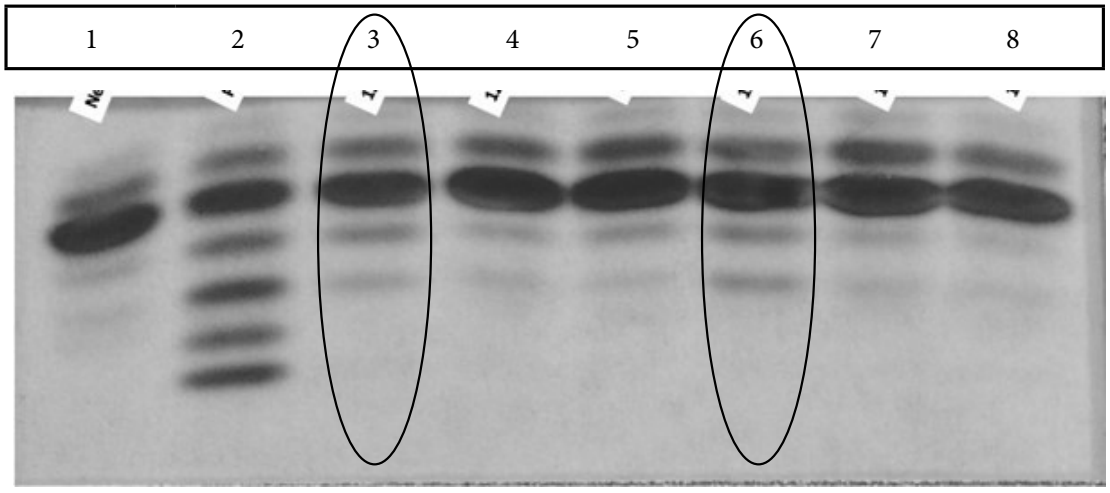
Half of our reported patients had an early presentation with hypotonia, hepatomegaly, elevated transaminases, mild hypoglycemia and various cerebral MRI abnormalities (cerebellar atrophy, mega cisterna magna, cortical atrophy and encephalomalacia). Eleven children had dysmorphic features, failure to thrive and neurological manifestation (seizures, mental retardation, ataxia). As additional clinical presentations there were abnormal coagulation, stroke-like episodes, cardiacarrhythmia, cutis laxa, inverted nipples, anemia, strabismus and nystagmus (figure 2).

Figure 2. Clinical manifestations of CDG suspected patients from Moldova



In all patients suspected for CDG the metabolic investigations (acid-base status, amino acids in blood and urine, organic acids in urine, acylcarnitine profile, coagulation studies) were performed for differential diagnosis. At the same time, the serum of suspected patients was analyzed by IEFT. In 37 cases there were obtained normal IEFT patterns, in other three patients the abnormal IEFT profile have been identified being suspected for CDG. In one positive IEFT patient there was detemined the galactose and galactitol in urine by ^{1}H NMR spectroscopy and these features were suggestive for Galactosemia, defined then by molecular analysis. In another one positive IEFT patient having the history of aversion to fructose-containing foods/sweets the diagnosis of Fructosemia was considered and then confirmed by DNA mutations analysis. These disorders are described having fals-positive IEFT results because of secondary abnormality of glycosylation. The last positive IEFT sample belonged to a boy born at term, from the 2nd normal pregnancy, born with normal body weight (3840g), in a non-consanguineous healthy family. From 5 months old he presented hepatomegaly (+5cm) and elevated transaminases, being breastfed at that time. At the moment of consultation, he was presenting with episodes of vomiting, frequent nosebleeds, hepatosplenomegaly, failure to thrive (short stature and less body weight), cutis laxa, angular front skull, unstable stool with frequent diarrhea, rotten teeth. He does not like sweets, fruits and many vegetables. The basic metabolic investigation was initiated with the following changes: anemia, neutropenia 28.4 % *(ref.val 30-75%)*, high Anion Gap -22.6 mmol/L (*ref. val. 7-16 mmol/L*), fasting hypoglycemia 70 mg/dL [3.85 mmol/L] (ref.val. 74-106 mg/dL), elevated transaminases, high TG [222 mg/dL *(ref.val <150 mg/dL)*] and low Iron [28.5 μg/dL *(ref.val 49-181* μg*/dL)*]. Amino acids in blood and urine and acylcarnitine profile were not suggestive. The abnormal coagulation was determined: high - C protein - 200% *(ref.val 70-130%)*, Factor X - 172% *(ref.val. 75-130%)*, fibrinogen - 5.4 g/L *(ref.val 2.0-4.0g/L)* and high number of platelet 520*10^{9}/L *(ref.val. 150-400)*. Abdominal ultrasound revealed liver steatosis of 3rd degree. As the consequence, α_1-antitrepsin deficiency, Gaucher Disease and Niemann-Pick A/B were excluded. Then, he was suspected for Congenital Disorders of Glycosylation and Fructose intolerance. His serum showed positive IEFT, but is necessary to make a differential diagnosis with Fructose intolerance because this pathology can determine a false-positive result of IEFT. First, it was tried fructose restricted diet for 1 month. As result, some clinical improvement like less nasal hemorrhages and no vomiting were observed, but liver ultrasound did not change. Then, the IEFT analysis was repeated and the type I patterns of carbohydrate-deficient transferrin identified on fructose-containing diet did not disappear after 4 weeks (fig 3). Another moment for diagnosis of CDG will be glycomics profile that is currently being performed in Translational Metabolic Laboratory Radboudumc, Nijmegen, Netherlands. But, a final diagnosis for Fructose intolerance can be established only by sequencing of ALDOB gene.

Figure 3. IEFT profiles: nr.3 - 1 mo after Fru-restricted diet, nr.6 – normal diet.



Discussions

CDG represent a group of monogenic pathologies with multisystem involvement predominantly neurologic. It is a challenge for a clinician due to his clinical chameleon manifestations that is why CDG is often underdiagnosed. In order to facilitate CDG diagnosis, there are reported some practical tools: (1) a list of clinical features strongly suggestive of a distinctive CDG; (2) a table of clinical, biochemical and laboratory findings reported in CDG, arranged per organ/system; (3) an overview of the affected organs/systems in each type of CDG; and (4) a diagnostic decision tree in face of a patient with a suspicion of CDG [1].

Taking into account multisystem impairment and clinical heterogeneity, the clinical criteria for suspicion of CDG must be very broad. Most important is to keep in mind a CDG in any unexplained syndrome, in particular when there is neurological involvement [1]. The „gold

standard" for diganosis of CDG is screening by IEFT. But, is very important in the diagnostic process to exclude the secondary glycosylation abnormalities such as fructosemia, galactosemia, alcoholism, polymorfism of transferrin, sever liver disease, etc. In case of differential diagnosis between Hereditary fructose intolerance (HFI) and CDG we can try the analysis of transferrin isoform by IEFT on a fructose-free diet. In the literature there are reported two cases, that following the fructose restriction diet, the type I patterns of carbohydrate-deficient transferrin detected on fructose-containing diet disappeared after 3-4 weeks. These cases illustrate that HFI may show a misleading clinical manifestation and the IEFT may give important diagnostic clue. However, the clinician must be careful, not to misinterpret the transferrin abnormal profile as CDG Ix, that why is needed the ALDOB mutation screening for HFI [13]. Therefore, in our patient which has been determined changes of transferin profile, HFI exclusion by ALDOB gene sequencing is crucial.

Conclusion

The CDG it is a group of rare diseases with multisystem involvement with a variety of symptoms that can determine missdiagnosis. Considering multisystem damage, there is recommended to suspect for CDG any unexplained neurological syndrome, particularly when there is associated with other organ disease, sometimes even without neurological involvement. In diagnosis of CDG by IEFT it is necessary to exclude the secondary abnormalities caused by Galactosemia, Fructosemia and other.

Bibliography:

1. Francisco R, Marques-da-Silva D, Brasil S, Pascoal C, dos Reis Ferreira V, Morava E, et al. The challenge of CDG diagnosis [Internet]. *Molecular Genetics and Metabolism Elsevier Inc;* 2019 p. 1–5. Available from: https://doi.org/10.1016/j.ymgme.2018.11.003.
2. Péanne R., P. de Lonlay, Foulquier F., Kornak U., et al. Congenital disorders of glycosylation (CDG): Quo vadis?, *Eur J. Med. Genet*. 2017; 1-14;
3. Freeze H. H., Chong J. C., Bamshad M. J., et al. Solving Glycosylation Disorders: Fundamental Approaches Reveal Complicated Pathways. *Am. J. of Hum. Genet.* 2014; 94, 161-175;
4. Jaeken J. Congenital disorders of glycosylation (CDG): it's (nearly) all in it! *J Inherit Metab Dis*. 2011 Aug;34(4):853-8. doi: 10.1007/s10545-011-9299-3;
5. Freeze H.H., Eklund E.A., Ng B.G., Patterson M.C. Neurological aspects of human glycosylation disorders. *Annu Rev Neurosci*. 2015 Jul 8;38:105-25. doi: 10.1146/annurev-neuro-071714-034019;
6. Marques-da-Silva D, Dos Reis Ferreira V, Monticelli M, et al. Lver involvement in congenital disorders of glycosylation (CDG). A systematic review of the literature. J Inherit Metab Dis. 2017;40:195–207
7. Rymen, D, Jaeken, Skin manifestations in CDG. J Inherit Metab Dis. 2014, 37; (5m): 699-708
8. Marques-da-Silva D., Francisco R., Webster D., Dos Reis Ferreira V., Jaeken J., Pulinilkunnil T.. Cardiac complications of congenital disorders of glycosylation (CDG): a systematic review of the literature. J Inherit Metab Dis. 2017 Sep;40(5):657-672. doi: 10.1007/s10545-017-0066-y
9. Monticelli M., Ferro T., Jaeken J., Dos Reis Ferreira V., Videira P. Immunological aspects of congenital disorders of glycosylation (CDG): a review. J Inherit Metab Dis. 2016 Nov;39(6):765-780.
10. Marquardt T, Denecke J. Congenital disorders of glycosylation: review of their molecular bases, clinical presentations and specific therapies. Eur J Pediatr, 2003;162(6):359–79.
11. Melanie A J, Madhuri R H. Congenital Disorders of Glycosylation. In: Denecke J.Molecular Pathology in Clinical Practice. Second Edition. USA: Springer International Publishing 2016:121-125.
12. Marklova E, Albahri Z. Screening and diagnosis of congenital disorders of glycosylation. Clin Chim Acta, 2007; 385(1-2):6–20.
13. Adamowicz M., Płoski R., Rokicki D., Morava E., Gizewska M., Mierzewska H., Pollak A., Lefeber D.J., Wevers R.A,. Pronicka E. Transferrin hypoglycosylation in hereditary fructose intolerance: using the clues and avoiding the pitfalls. *J Inherit Metab Dis*. 2007 Jun;30(3):407.