https://doi.org/10.52418/moldovan-med-j.64-4.21.09
UDC: 616-008.9-053.1-07

The screening by isoelectric focusing of transferrin for the diagnosis of congenital disorders of glycosylation

***Daniela Blanita, Chiril Boiciuc, Doina Turcan, Victoria Sacara, Natalia Usurelu**

Laboratory of Prevention of Hereditary Pathologies, Centre of Reproductive Health and Medical Genetics
Institute of Mother and Child, Chisinau, the Republic of Moldova

Authors' ORCID iDs, academic degrees and contributions are available at the end of the article

*Corresponding author – Daniela Blanita, e-mail: blanita.daniela@gmail.com
Manuscript received July 3, 2021; revised manuscript October 01, 2021; published online October 12, 2021.

Abstract

Background: Congenital Disorders of Glycosylation (CDG) are a group of inherited metabolic disorders caused by the defect in various steps in the biosynthesis of glycoproteins and other glycoconjugates.
Material and methods: 40 patients under clinical suspicions for CDG at the Institute of Mother and Child were examined by isoelectric focusing of transferrin (IEFT) in collaboration with RadboudUMC, Netherlands and U.S.A. The spectrum of clinical presentations of these patients was multisystem damage, predominantly neurological manifestations.
Results: Most of the patients (55%) had early neurological manifestations from the birth, such as hypotonia, psychomotor disability, cerebral MRI abnormalities, seizures (25%), cutis laxa (17.5%), total alopecia (2.5%), abnormal fat pads (2.5%), myopia (7.5%), nystagmus (5%), strabismus (2.5%), stroke-like episodes (2.5.%), ataxia (7.5%), abnormal coagulation (10%), hepatomegaly (35%) and liver cirrhosis (2.5%). Serum samples analyzed by IEFT showed the results: 37 normal, 2 questionable and 1 abnormal patterns. Two samples questionable belongs to the patients with Galactosemia and Fructosemia, which give the false-positive results. The last positive sample is performed additionally for glycomics profiling. In some cases, with IEFT negative profile was performed genetic test and were diagnosed other diseases, mimicking CDG, such as: NARP syndrome, late diagnosed PKU, GSD, Manosidoses, Prader-Willi Syndrome and chromosomal aberrations.
Conclusions: The CDG is a rare metabolic disease with multisystem impairment and variety of symptoms which determine overlapping of phenotype with other genetic disorders. The process of diagnosis is very complex and can take several years.
Key words: congenital disorders of glycosylation, multisystem affections, isoelectric focusing of transferring.

Cite this article
Blanita D, Boiciuc C, Turcan D, Sacara V, Usurelu N. The screening by isoelectric focusing of transferrin for the diagnosis of congenital disorders of glycosylation. *Mold Med J.* 2021;64(4):50-54. https://doi.org/10.52418/moldovan-med-j.64-4.21.09.

Introduction

Congenital disorders of glycosylation are a rare group of inherited metabolic disorders caused by genetic defects in various steps in the biosynthesis of glycoproteins and other glycoconjugates. First case of Congenital Disorders of Glycosylation (CDG) was described in 1980 by Jaeken J. at the twin-sister with marked psychomotor retardation [1]. The incidence and prevalence of CDG have not been well established because of clinical heterogeneities and complexity of diagnosis. Currently, more than 140 different types of CDG are reported in over 1350 patients diagnosed at molecular level. According to the latest reports, CDG is classified in protein N- or protein O-glycosylation defects, glycosphingolipids and glycosylphosphatidylinositol anchor glycosylation defects, and multiple glycosylation pathway defects [2]. The most common group of the pathologies are N-glycosylation disorders responsible for more than 70 types of CDG. PMM2-CDG (CDG-1a) is the most frequent diagnosed type of CDG which is identified in 62% of the patients with the ranges from 1/20000 in Dutch population and 1/77000 in Estonia based on isolated reports [3, 4]. This ultra-rare group of pathologies shows an exponential growth due to the development of sequencing technologies, so that in 2013 every 11 days a new type of CDG was confirmed and other 5 new forms in the first half of 2017 have been described [3]. The glycoproteins exist in whole body and the incorrect process of protein glycosylation disrupts the function of many organs and tissues leading to multisystem impairment and clinical heterogeneity involving 80% of neurological manifestations, 22% – jg-hepatic disorders, 20% – cardiac damage, 20% – dermatological and 10% – immunological troubles and others [5-9]. Often, the variability of clinical manifestation is mimicking other pathologies which determine the non-recognition and underdiagnosis of CDG. The first step in diagnosis of this group of pathologies is the isoelectric focusing of transferrin (IEFT) – a screening method considered as a "gold standard" in the diagnosis of CDG. The abnormal isoelectric profile of transferrin may divide the CDG into two large groups – CDG I and CDG II. During the process of the diagnosis the high necessary moment is to exclude the secondary abnormalities of glyco-

sylation leading to false-positive profiles as in Fructosemia, Galactosemia, severe liver disease, alcoholism and others. As a final step in research of CDG the clinical glycomics and genetic diagnosis are used.

Material and methods

Following the medical genetic counseling in the Laboratory of Prevention of Hereditary Pathologies of the Centre of Reproductive Health and Medical Genetics (the Institute of Mother and Child, Chisinau) there were collected 40 samples of serum from suspected for CDG patients. The children with multisystem impairment and a predominantly undefined diagnosis associated with other suspicion criteria for CDG (tab. 1) have been selected. To characterize CDGs forms and differentiate other metabolic errors which mimic CDG, the basic first- and second-line metabolic work-up has been done. For excluding chromosomal aberrations, the karyotype and CGH-array was performed. In order to complete the metabolic investigations the spectrum of urinary organic acids was analyzed by ^{1}H-NMR spectroscopy at "Petru Poni" Institute of Macromolecular Chemistry of Romanian Academy, Iasi, Romania. The screening of all patients with clinical phenotype compatible with CDG was performed by serum transferrin isoelectric focusing in collaboration with Translation Metabolic Laboratory.

Table 1. Suspicion criteria for CDG included in the study

Suspicion criteria for CDG
Age from 3 months to 18 years
Psychomotor disability
Hypotonia
Failure to thrive
Seizures
Dysmorphic features excluding chromosomal aberrations

Radboundumc, Nijmegen, The Netherland and Mayo clinic, U.S.A. In some cases with very specific clinical manifestations the genetic testing was done by genes panel or Whole Exome Sequencing in collaboration with partners from Cytogenomic Medical Laboratory, Bucharest, Romania.

Results

The cohort of patients selected for screening by IEFT were children of various age (2mo-15y) with an unexplained multisystem involvement, predominantly including nervous system. Most of the patients (55%) had early neurological manifestations from birth: hypotonia, psychomotor disability, brain MRI abnormalities as cerebellar and/or cortical atrophy, hypogenesia of corpum callosum, mega cisterna mag-

Table 2. Neurological manifestation in the suspected CDG patients

Serum	Hypotonia	IEFT profile	Cerebelar MRI					Neurological finding						Serum	Hypotonia	IEFT profile	Cerebelar MRI					Neurological finding					
			Hypogenesia of corpus calosum	Cerebelar atrophy	Cortical atrophy	Encephalomalacia	Mega cisterna magna	Stroke-like episodes	Strabismus	Nistagmus	Seizures	Ataxia	Mental retardation				Hypogenesia of corpus calosum	Cerebral atrophy	Cortical atrophy	Encephalomalacia	Mega cisterna magna	Stroke-like episodes	Strabismus	Nistagmus	Seizures	Ataxia	Mental retardation
S1	x	-	x						x		x		x	S21		+											
S2	x	-											x	**S22**	x	-			x								
S3	x	-												**S23**	x	-									x		x
S4	x	-										x	x	S24		-									x		
S5		+												**S25**	x	-	x		x					x	x	x	
S6	x	-	x										x	**S26**	x	-											
S7		-											x	**S27**	x	-						x		x		x
S8	x	-											x	**S28**	x	-								x		
S9	x	-	x				x						x	**S29**	x	-						x	x			
S10	x	-					x						x	**S30**	x	-					x		x		x	
S11	x	-												**S31**	x	-	x		x				x			x
S12	x	-											x	**S32**	x	-										
S13	x	-												**S33**	x	-				x		x				
S14	x	-												S34		-								x		
S15	x	-				x					x		x	**S35**	x	+										
S16		-				x					x			S36		-								x		
S17		-							x					**S37**	x	-							x			x
S18	x	-	x							x			x	**S38**	x	-										
S19	x	-		x			x				x		x	S39		-								x		
S20	x	-		x								x	x	**S40**	x	-									x	x

na, encephalomalacia and leukomalacia (tab. 2), reported in other described cases of CDG [10, 11]. The seizures associated with abnormal EEG were present at 25% of patients, in 3 children the abnormal EEG was identified without clinical convulsions.

The phenotype evaluation of investigated patients paid attention to dysmorphic features as inverted nipples (15%), microcephaly (7.5%) and "doll face" (5%). The dermatological examination gave importance in presence of cutis laxa (17.5%), total alopecia (2.5%), ichthyoid exfoliative dermatitis (2.5%) and abnormal fat pad (2.5%). The cardiological involvement as dilative (2.5%) and hypertrophic (5%) cardiomyopathy was found.

The important clinical signs directly suggest CDG were responsible for the failure to thrive and growth retardation which have been found in this cohort respectively in 17.5% and 20% of patients. Other affected system in CDG is ophthalmic and the manifestations detected in these subjects were: myopia (7.5%), nystagmus (5%), strabismus (2.5%) and optic atrophy (2.5%). The impairment of liver in CDG is one of the most important clue to suspect these pathologies and in the present studied cases hepatomegaly was in 35% and liver cirrhosis in 2.5 %. Other symptoms of no less importance presented by our patients were hypoglycemia (16%), stroke-like episodes (2.5%), ataxia (7.5%) and abnormal coagulation (10%).

The screening by IEFT was made to all patients who were suspected for CDG. 37 analyzed serums had no modification on IEFT, but in other 3 samples a positive transferrin profile on IEFT was identified, that allowed to suspect CDG. In all 3 cases were recommended to exclude secondary abnormalities of glycosylation. In S-5, one of the positive IEFT patients presented hepatomegaly, failure to thrive, high transaminases, high postprandial lactate, cataracts, galactose and galactitol in urine by ^{1}H NMR spectroscopy which were determined by metabolic work-up – features suggestive for Galactosemia, confirmed then by the mutation P.E203L/E203L in homozygous status in *GALT* gene. So, the Galactosemia is reported as a condition leading to false-positive IEFT profile [12] and should be excluded obligatory in suspected CDG subjects. The second abnormal profile on IEFT was in S-21, but the molecular analysis in correlation with phenotype of patient revealed that positive profile of transferrin was caused by fructose intolerance as a disease that determines secondary abnormality at IEFT, as well. The last positive serum S-35 was one of the patients who manifested hepatomegaly, elevated transaminases, cutis laxa, failure to thrive, dysmorphic features, hypoglycemia, unstable stool with frequent diarrhea, abnormal coagulation. The IEFT profile revealed the presence of disialo- and asialotransferrin that leads to the suspicion for CDG type I (fig. 1).

Initially, there were excluded the secondary abnormalities of glycosylation as Galactosemia and Fructosemia. His urinary NMR profile was negative for galactose and galactitol. The used fructose-free diet did not change the IEFT profile, confirmed by ALDOB negative result then. Next steps

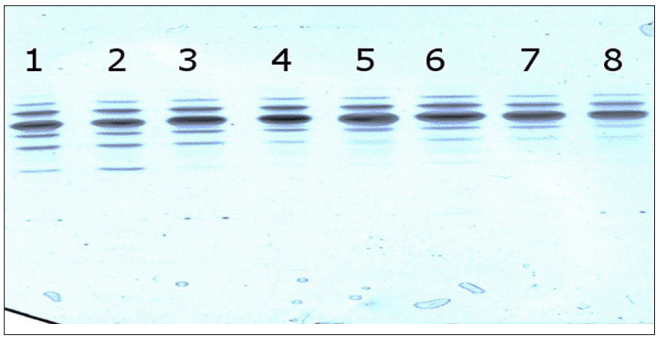

Fig. 1. IEFT profile: 1-3 positive profile, 4-5 negative profile

for the research and diagnosis of CDG in this case there will be clinical glycomics, that comprises a spectrum of different analytic methodologies which provide insights into the mechanisms of glycosylation that will be performed in collaboration with RadboudUMC, Nijmegen, The Netherlands [13].

In the rest of reported cases with normal IEFT the diagnosis tests continued to reveal other diseases those that mimic CDG (tab. 3). For the first, in the cases with severe hypotonia SMA and Pompe Disease were excluded. After metabolic investigations in some cases there were found hyperlactacidemia, hypoglycemia, hyperammonemia, high Anion Gap and acidosis that is highly suggestive for the inborn errors of metabolism, especially for glycogen storage disorders that were diagnosed in 2 cases. The diagnosis of other groups of inborn errors of metabolism will involve the specific tests which will be performed in future.

Many genetic tests were used to differentiate other genetic disorders mimicking CDG. As a consequence, in the TL1gene of mitochondrial genome was revealed the presence of m.3243A>G mutation associated with Leigh Syndrome.

This is a mitochondrial disorder that usually overlaps with clinical features of CDG.

In other two cases (S 9 and S 12), the genetic tests revealed mutations in PAH gene, that confirmed PKU. The studied patients with PKU developed a non-classical more severe phenotype of disease, possibly due to late diagnosis.

In those patients with unspecific for CDG dysmorphic features and severe hypotonia, the molecular karyotype (CGH array) was performed. So, in 1 case a pathogenic variant of microdeletion on chrs 16 p11.2 was found and in 2 other cases the Prader Willi Syndrome patterns were identified. In one case with severe development delay, seizures, tetraparesis, dysmorphic features and osteoporosis, the enzyme assay suggested the presence of Mannosidosis, which subsequently required genetic confirmation.

At the same time, the negative profile of IEFT does not exclude a CDG form and it is necessary to consider other types of glycosylation troubles as O-, GPI-anchor-, lipid- and multiple defects of glycosylation needing to be investigated by other specific tests.

Table 3. The diseases that mimicked CDG in reported cases

No	Clinical manifestation	IEFT profile	Other disease that mimic CDG
1.	Nephrosclerosis, hypertension, failure to thrive, periodic hypoglycemia, elevated transaminases	Negative	Mutation in SLC9A3R1 gene c.328C<G
2.	Psychomotor retardation, hypotonia,, cutis laxa, dysmorphic features, episodic opistatonus, knee joint contractures, hypogenesia of corpum callosum	Negative	Leigh Syndrome
3.	Hypotonia from birth, inverted nipples, fatigue, mild mental retardation, speech disorders, amenorrhea, myopia, hypothyroidism	Negative	Prader Willi Syndrome
4.	Severe neonatal hypotonia, hepatomegaly, fatigability, hypothermia, passivity drowsiness, psychomotor delay	Negative	Prader Willi Syndrome
5.	Psychomotor delay, hypotonia, ataxia, hepatomegaly, fatigability, general weakness.	Negative	Microdeletion on crs 16 p11.2
6.	Failure to thrive, disliking of fruits, some vegetables and sweets with vomiting, fasting hypoglycemia, hepatomegaly	Positive	Fructose intolerance
7.	Prolonged jaundice, high transaminases, high postprandial lactate, cataracts, hypotonia, hepatomegaly, failure to thrive.	Positive	Galactosemia
8.	Mental retardation, cutis laxa, stereotypic behavior, inverted nipples, dysmyelination on brain MRI	Negative	PKU
9.	Short stature, total alopecia, failure to thrive, feeding difficulty, dsymorphic facial features, lymphedematous hands and foots	Negative	PKU
10	Severe hypoglycemia, hypotonia, psychomotor retardation, seizures, micorcephaly, hepatomegaly, elevated transaminases	Negative	GSD
11	Generalized hypotonia, cutis laxa, hepatomegaly, mild elevated transaminases, osteoporosis, born with multiple congenital anomalies, tetraparesis, developmental delay	Negative	? Mannosidosis

Discussion

CDG are a group of rare disorders with clinical heterogeneity and the suspicions criteria for CDG should be very broad. It is recommended to suspect CDG in any unexplained affected patients, predominantly with neurological manifestations [13]. The variability of clinical symptoms is often a challenge for clinicians and can lead even to the underdiagnosis of this group of pathology that is why the real incidence of CDG is actually unknown. At the same time, the multisystem clinical involvement can mimic other genetic pathologies as: chromosomal aberration, mitochondrial disorders, lysosomal disorders and etc. Usually, clinical phenotype of the CDG, overlaps with mitochondrial disorders because both are multisystem disorders presented from birth with high mortality. The recent statistic data show that about 10% to 20% of individuals with either mitochondrial disease or CDG die in the first 2 to 4 years of life [14]. Searching the primary defect in multisystem affected patients is often unsuccessful, and mitochondrial patients could remain as suspected for CDG ones [15]. In the investigated group a suspected for CDG patient was diagnosed with a mitochondrial disorder – Leigh Syndrome (S-1, tab. 3).

A variety of complex tests is necessary to establish a diagnosis and to describe a subtype of CDG and the "gold standard" to first recognize CDG is the screening through IEFT, first introduced in 1984 by Jaeken J. [16]. This method allows diagnosing predominantly the disorders of N-glycosylation, because transferrin is N-glycosylated. The diagnosis way is followed by many steps based on the biochemical tests, enzyme assay and genetic analysis either by single gene, CDG panel or WES/WGS and clinical glycomics as well [2, 10]. Actually, to define a CDG diagnosis takes more time and it is more expensive than IEFT screening [11]. At the same time, the negative profile of IEFT does not exclude a CDG form and it is necessary to consider other types of glycosylation troubles as O-, GPI-anchor-, lipid- and multiple defects of glycosylation needing to be investigated by other specific tests.

However, the percentage of solved cases constituted about 30%, that is still quite low, which is caused by: either the lack of sequence coverage of the variant, or disease causes outside the coding sequences, or the presence of too many variants of unknown significance [2]. That is why, the important connection of genomics with functional *-omics* methodologies in the diagnosis of metabolic disorders is recognized more and more. For CDG, many cases were unsolved until the inclusion of glycomics into clinical practice, to present the functional defect [2]. These moments, revealed the complexity of diagnosis of CDG and at the same time this process involves a multidisciplinary team, multiple resources, and the implications of various international research groups as well.

Conclusions

Taking into account the fact that CDG is a group of ultrarare disorders with multisystem involvement and heterogeneous phenotype, the screening criteria for suspected CDG should be very broad. Differential diagnosis of this group of disorders should be very detailed and meticulous, because the phenotype most often mimics other genetic disorders. Because CDG is a new exponential group of disorders, it is recommended in the process of diagnoses of CDG to get involved a multidisciplinary researchers around the world and to develop the screening diagnosis in each country.

References

1. Jaeken J, Vanderschueren-Lodeweyckx M, Casaer P, Snoeck L, Corbeel L, Eggermont E, Eeckels R. Familial psychomotor retardation with markedly fluctuating serum proteins, FSH and GH levels, partial TBG deficiency, increased serum arylsulphatase A and increased CSF proteins: a new syndrome? Pediatr Res. 1980;14(2):179. https://doi.org/10.1203/00006450-198002000-00117.
2. Abu Bakar N, Lefeber DJ, van Scherpenzeel M. Clinical glycomics for the diagnosis of congenital disorders of glycosylation. J Inherit Metab Dis. 2018;41(3):499-513. https://doi.org/10.1007/s10545-018-0144-9.
3. Jaeken J, Peanne R. What is new in CDG? J Inherit Metab Dis. 2017;40(4):569-586. doi: 10.1007/s10545-017-0050-6.
4. Francisco R, Marques-da-Silva D, Brasil S, Pascoal C, dos Reis Ferreira V, Morava E, et al. The challenge of CDG diagnosis. Mol Genet Metab. 2019;126(1):1-5. https://doi.org/10.1016/j.ymgme.2018.11.003.
5. Marques-da-Silva D, Dos Reis Ferreira V, Monticelli M, et al. Liver involvement in congenital disorders of glycosylation (CDG). A systematic review of the literature. J Inherit Metab Dis. 2017;40(2):195-207. doi: 10.1007/s10545-016-0012-4.
6. Freeze HH, Eklund EA, Ng BG, Patterson MC. Neurological aspects of human glycosylation disorders. Annu Rev Neurosci. 2015 Jul 8;38:105-25. doi: 10.1146/annurev-neuro-071714-034019.
7. Rymen D, Jaeken J. Skin manifestation in CDG. J Inherit Metab Dis. 2014;37(5):699-708. doi: 10.1007/s10545-014-9678-7.
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 systematic review of the literature. J Inherit Metab Dis. 2016 Nov;39(6):765-780. doi: 10.1007/s10545-016-9954-9.
10. Paprocka J, Jeleza-Stanek A, Tylki-Szymanska A, Grunewald S. Congenital disorders of glycosylation from neurological perspective. Brain Sci. 2021 Jan 11;11(1):88. doi: 10.3390/brainsci11010088.
11. Van Scherpenzeel M, Willems E, Lefeber DJ. Clinical diagnostics and therapy monitoring in the congenital disorders of glycosylation. Glycoconj J. 2016;33(3):345-358. doi: 10.1007/s10719-015-9639-x.
12. Maratha A, Colhoun HO, Knerr I, Coss KP, Doran P, Treacy EP. Classical galactosaemia and CDG, the N-Glycosylation interface. A Review. JIMD Rep. 2017;34:33-42. doi: 10.1007/8904_2016_5.
13. Jaeken J. Congenital disorders of glycosylation (CDG): it's (nearly) all in it! J Inherit Metab Dis. 2011;34(4):853-858. doi: 10.1007/s10545-011-9299-3.
14. Funke S, Gardeitchik T, Kouwenberg D, et al. Perinatal and early infantile symptoms in congenital disorders of glycosylation. Am J Med Genet A. 2013;161a(3):578-84. https://doi.org/10.1002/ajmg.a.35702.
15. Briones P, Vilaseca MA, Garcia-Silva MT, Artigas J. Congenital disorders of glycosylation (CDG) may be underdiagnosed when mimicking mitochondrial disease. Eur J Paediatr Neurol. 2001;5(3):127-131. doi: 10.1053/ejpn.2001.0483.
16. Jaeken J, Van Eijk HG, van der Heul C, Corbeel L, Eeckels R, Eggermont E. Sialic acid-deficient serum and cerebrospinal fluid transferrin in a newly recognized genetic syndrome. Clin Chim Acta. 1984;144(2-3):245-247. doi: 10.1016/0009-8981(84)90059-7.

Authors' ORCID iDs and academic degrees

Daniela Blanita, MD, PhD Applicant – https://orcid.org/0000-0001-7736-3406
Chiril Boiciuc, BioD, PhD Applicant – https://orcid.org/0000-0002-7273-2492
Doina Turcan, BioD, PhD Applicant – https://orcid.org/0000-0002-8571-0524
Victoria Sacara, BioD, BioPhD, Associate Professor – https://orcid.org/0000-0001-9200-0494
Natalia Usurelu, MD, PhD, Associate Professor – https://orcid.org/0000-0001-8685-3933

Authors' contributions

DB collected and interpreted the data. DB, CB, DT, VS and NU analysed the result of screening by IEFT. NU revised the manuscript critically. All the authors approved the final version of the manuscript.

Funding

This study was supported by *Nicolae Testemitanu* State University of Medicine and Pharmacy of the Republic of Moldova. The trial was the authors' initiative. The authors are independent and take responsibility for the integrity of the data and accuracy of the data analysis.

Ethics approval and consent to participate

This research project was approved by the Research Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy (Protocol No 48 of 03.07.2019).

Conflict of interests

No competing interests were disclosed