The Turkish Journal of Pediatrics 2018; 60: 540-546
DOI: 10.24953/turkjped.2018.05.011

Original

Folate deficiency in patients with classical galactosemia: A novel finding that needs to be considered for dietary treatments

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Received: 27th December 2017, Revised: 8th February 2018, 10th April 2018, Accepted: 21st April 2018

SUMMARY: Çelik M, Özgün N, Akdeniz O, Fidan M, Tüzün H, İpek MŞ, Emecan M, Eminoğlu FT. Folate deficiency in patients with classical galactosemia: A novel finding that needs to be considered for dietary treatments. Turk J Pediatr 2018; 60: 540-546.

The objectives of the study were to assess folate deficiency in patients with classic galactosemia, and to determine whether folic acid supplementation has an effect on galactose-1-phosphate uridyltransferase enzyme activity. Sixty-one newborn infants diagnosed with classic galactosemia between 2010 and 2017 were retrospectively evaluated. Within this group, 48 patients with Q188R homozygous mutation alone were enrolled into the study. Serum folate concentration was studied using chemiluminescence; and in folate deficient patients, galactose-1-phosphate uridyltransferase measurements before and after folic acid supplementation (100 μg/day folic acid for 30 days) were performed using an enzymatic calorimetric measurement technique based on kinetics. The serum folate level was low (<4 ng/ml) in 12 patients (25%). The galactose-1-phosphate uridyltransferase enzyme activity after folic acid supplementation was significantly higher than the values before folic acid supplementation (1.00±0.19 U/g Hb vs. 0.74±0.23 U/g Hb, p<0.05); but was still less than the normal levels. Folate deficiency, most likely due to poor dietary intake, may develop in pediatric patients with classical galactosemia, and folic acid should be supplemented. Folic acid supplementation appears to have a low, but statistically significant, effect on galactose-1-phosphate uridyltransferase enzyme activity, but comprehensive research is needed to clarify whether there is any clinical significance.

Key words: classic galactosemia, folate deficiency, folic acid, galactose-1-phosphate uridyltransferase enzyme.

Classic galactosemia (CG) (type 1 galactosemia, OMIM #230400) is an autosomal recessive inherited disease associated with a deficiency in the activity of galactose-1-phosphate uridyltransferase (GALT, EC 2.7.7.12), which is the second enzyme in the Leloir pathway that catalyzes the conversion of galactose to glucose.[1] CG has a high mortality rate unless treated properly and rapidly.[1] The first principle of acute treatment is the initiation of a galactose-free diet and supportive treatment. The only treatment for CG currently is a galactose (and lactose)-free diet.[2,3]

The prescribed dietary treatment varies widely around the world. Although current recommendations are to maintain a galactose-restricted diet lifelong, controversy surrounding the proper dietary treatment is still ongoing. The most discussed topic in this field is that relaxation of the galactose-restricted diet may be considered as the patients get older.[2,4-6] Prolonged galactose restriction may predispose patients to some nutritional deficiencies. Generalized osteopenia is a typical outcome and is thought to be a consequence of not only mineral deficiency but also, possibly,

unknown intrinsic factors.[7,8] Recently, more frequent observation of certain gastrointestinal symptoms in patients with CG was also reported as a result of a galactose-restricted diet.[9]

Although infant formulas with a very limited amount of galactose are widely available, most of these formulas do not meet the daily requirement of 80 μg of folate recommended for infants.[10] After the introduction of solid foods, patients may not get enough folate from limited diets in which the allowed foods contain trace amounts of galactose, especially under a rigorous dietary galactose restriction. Therefore, patients with CG may be at risk for the development of folate deficiencies.

Some experimental new treatment strategies for prevention of long-term complications have been investigated. One strategy is to enhance residual GALT enzyme activity. Recently, trace residual GALT activity was shown to be associated with both improved and ovarian outcomes, suggesting that residual GALT activity may influence the severity of long-term complications in CG.[11,12] In an animal study, it was reported that residual GALT enzyme activity can be enhanced by folic acid.[13]

Considering that most of the available commercial lactose-free formulations and foods in the CG dietary treatment regimen may not meet the daily folate requirement, in this study, we aimed to assess folate deficiency in patients with CG, and to determine whether folic acid supplementation has an effect on residual GALT enzyme activity.

Material and Methods

This study was conducted at the Division of Neonatology, Diyarbakir Children's Hospital, Turkey. Sixty-one newborn infants who were admitted to the hospital between 2010 and 2017 and diagnosed with CG based on the classic findings of low GALT enzyme activity (<3 U/g Hb), and/or positive mutation analysis were retrospectively evaluated. Regarding genetic homogeneity, 48 patients with Q188R homozygous mutation alone were enrolled in the study. Eleven patients without mutation analysis data and two patients with the 314D/N314D mutation were excluded. The complete blood count, and serum folic acid and vitamin B12 levels of all patients were obtained and studied using an automated cell counter and chemiluminescence, respectively. A serum folic acid level of less than 4 ng/ml was considered to indicate a folate deficiency. All patients with folate deficiency were treated with 100 μg/day folic acid for 30 days. Before and after treatment, the total galactose, free galactose, Gal-1-P, and GALT enzyme activity levels were recorded. The demographic features, laboratory results, and enzyme activity levels were obtained from the patient records.

GALT measurements were performed based on kinetics using an enzymatic colorimetric measurement technique based on the consumption of uridine-5' diphosphoglucose (Modified Beutler method), and total and free galactose levels were recorded using a colorimetric microassay technique (Modified Diepenbrock method). The GALT mutation analysis was obtained using the tetra-primer Amplification-Refractory Mutation System-Polymerase Chain Reaction (PCR) method. Wild-type and mutant alleles were amplified simultaneously by PCR in the presence of an internal control; amplicons were separated by agarose gel electrophoresis according to amplicon length. All tests were performed in the same laboratory.

The study was approved by Clinical Research Ethics Board (Registration Number: no: 2015-80 12148/40). Written informed consent was obtained from the all patients' parents.

Statistical analysis

Statistical analyzes were performed using the SPSS for Windows (version 15.0) statistical package. The Shapiro-Wilk test was performed to examine the distribution of data. Paired sample student's t-test was used to compare the mean values of variables before and after folic acid supplementation. A two-tailed p-value of <0.05 was considered to be statistically significant. Parametric continuous variables are expressed as mean±standard deviation, nonparametric continuous variables are expressed as the median (interquartile range), and categorical variables are expressed as numbers (%).

Results

Folate deficiency was found in 12 patients (25%); only one patient concomitantly had

Table I. Demographic and Clinical Characteristics of the Patients.

Patients/ Gender	Gestational age (week)	Birth weight (gram)	Parental consanguinity	Admission day	Presenting complaints	tGal (mg/dl)	fGal (mg/dl)	Gal-1-P (mg/dl)	GALT (U/g Hb)	Cataract
1/M	39	2,460	+	5	jaundice	77.9	51.7	26.2	1.24	-
2/F	40	3,000	+	7	jaundice	172	126	46	1.13	+
3/M	39	3,200	-	7	jaundice	266	216	50	1.27	-
4/F	39	3,600	+	9	convulsions	58.2	44.1	14.1	1.14	-
5/F	39	3,000	+	7	jaundice	273.6	190.4	83.2	1.68	-
6/F	39	2,350	+	6	jaundice	295	273	22	0.83	+
7/F	40	3,400	+	13	jaundice, HM, bleeding	101	83	18	0.5	-
8/F	38	2,550	+	5	jaundice	283	234	49	1.42	-
9/M	39	3,700	-	6	jaundice	164	147	17	1.04	-
10/M	39	3,500	-	7	jaundice	305.3	290.5	14.8	1.48	-
11/F	40	2,500	+	24	jaundice, HM, bleeding	300	282	18	1.57	+
12/M	37	2,980	-	6	Bleeding, vomiting	168	130	38	1.6	-

Gal-1-P: galactose-1-phosphate, GALT: galactose-1-phosphate uridyltransferase, fGal: free galactose, F: female, Hb: hemoglobin, HM: hepatomegaly, M: male, tGal: total galactose.

a vitamin B12 deficiency (76 ng/ml, normal range: 179-883 ng/ml) and anemia (hemoglobin was 8.1 g/dl). The demographic and clinical features of the patients are shown in Table I.

GALT activity at the time of diagnosis was 1.24±0.34 U/g Hb, the Gal-1-P level was 33.03±20.93 mg/dl, the free galactose level was 172.3±88.2 mg/dl, and the total galactose level was 205.3±92.6 mg/dl.

At the time of the study, the median age of the patients was 12 months (7.25-31.75), and the folic acid levels were 3.37±0.36 ng/ml. The GALT, Gal-1-P, total galactose, and free galactose levels before and after folic acid treatment are shown in Table II.

No statistically significant difference in the levels of total and free galactose was determined before versus after folic acid treatment. However, the GALT enzyme activity was 0.74±0.23 U/g Hb before folic acid treatment and significantly increased to 1.00±0.19 U/g Hb at the end of the treatment (p=0.002). The Gal-1-P level was 1.38±0.73 mg/dl before the treatment and significantly increased to 2.11±0.92 mg/dl at the end of the treatment (p=0.009) (Tables II).

Discussion

The objectives of this study were to assess folate deficiency in patients with CG, to determine whether folic acid supplementation influences GALT enzyme activity in folate deficient patients. The results demonstrated that about 25% of the patients in the study group had a folate deficiency, and that folic acid treatment over a month has a low, but statistically significant, effect on residual GALT enzyme activity in these patients.

In English literature, there is scant information on folic acid and galactose metabolism or galactosemia. As far as we know, folate deficiency in patients with CG has not been evaluated yet. The current state of knowledge about the relationship between folic acid levels and CG is based only on an experimental study, which found that pharmacological doses of folic acid might increase GALT activity in rats.[13] Therefore, the findings in our study may open up new avenues of investigation into the pathophysiology of CG and create possibilities for therapeutic intervention.

Folate deficiency in the study group may be due to the fact that the folic acid content in commercially available lactose-free formulations does not meet the daily folic acid requirement, since the folic acid content of lactose-free formulas is lower than that of standard infant formulas (e.g., 100 ml of Bebelac LF® provides 8.9 mg of folic acid whereas 100 ml of Bebelac Gold 1® provides 13 mg). There are no lactose-free formulas with high folic acid content in our country. For CG patients, lactose-free formulation is the only component of dietary therapy during infancy. After the infancy period, a limited nutrient diversity due to very strict dietary requirements may contribute to the development or continuity of folate deficiency. Naturally rich sources of folic acid such as legumes, organ meats and some grain products are not permitted. Highly restrictive diets in infancy may also lead to a reduction in fruit and/or vegetable consumption.[2,14] In addition to poor folic acid intake, the alteration of the gut microbiome due to restriction of galactose-containing foods or the effect of defective glycosylation on the mucosal layer of the gut may have an impact on micronutrient bioavailability.

The recommended dietary intake of folic acid differs depending on age; 80 μg/day for infants aged 6-12 months, 150 μg/day for children aged 1-3 years, and 400 μg/day for adults.[10] Folate deficiency in infants with CG can be prevented by increasing the folic acid content of lactose-free formulations. For older patients, folate deficiency can be prevented by including good sources of folic acid into the dietary list while avoiding foods that are the major sources of galactose. If these measures are not possible, serum folate levels should be checked at regular intervals as part of the follow-up schedule and, if necessary, pharmacological treatment should be considered. The finding that the frequency of folate deficiency is as high as 25% in the study group indicates that measures should be taken into consideration for this population, similar to the measures prescribed for pregnant or breastfeeding women.[15]

Another interesting finding of our study is that folic acid supplementation might affect GALT enzyme activity. A longer period of folic acid supplementation might increase GALT enzyme activity to a higher level. Although the GALT

Table II. The Changes in Levels of the Most Relevant Biomarkers and GALT Enzyme Activity with Folic Acid Treatment.

Patient	Age of the patients (month)	Folic acid (ng/ml)	Before/after folic acid treatment tGal (mg/dl)	Before/after folic acid treatment fGal (mg/dl)	Before/after folic acid treatment Gal-1-P (mg/dl)	Before/after folic acid treatment GALT (U/g Hb)
1	7	3.2	3.6 / 4.7	2.5 / 2.5	1.1 / 2.2	0.67 / 0.84
2	10	2.8	5.2 / 3.8	2.7 / 1.8	2.5 / 2.0	0.67 / 0.89
3	14	3.9	3.2 / 2.6	1.9 / 0.3	1.3 / 2.3	1.02 / 1.07
4	28	3.5	2.8 / 2.9	2.5 / 2.2	0.3 / 0.7	0.5 / 1.02
5	59	3.5	3.7 / 5.6	2.1 / 2.3	1.6 / 3.3	0.81 / 1.03
6	47	3.8	2.2 / 3.7	1.8 / 1.4	0.4 / 2.3	0.64 / 0.81
7	6	3.2	2.5 / 3.0	1.3 / 2.0	1.2 / 1.0	1.13 / 1.02
8	18	3.7	3.3 / 3.4	1.7 / 1.3	1.6 / 2.1	0.33 / 0.85
9	8	3.5	2.6 / 2.1	2.1 / 0.8	0.5 / 1.3	0.55 / 1.02
10	33	3.7	3.6 / 4.0	1.9 / 1.9	1.7 / 2.1	0.71 / 1.14
11	8	2.9	2.5 / 3.3	1.1 / 1.4	2.0 / 1.9	1.01 / 1.48
12	6	3.1	2.2 / 5.5	1.7 / 1.4	2.4 / 4.1	0.8 / 0.77
	12 (7.25-31.75)	3.40±0.36	3.12±0.85 / 3.72±1.09	1.94±0.48 / 1.61±0.64	1.38±0.73 / 2.11±0.92	0.74±0.23 / 1.00±0.19
p			0.125	0.110	0.009	0.002

Gal-1-P: galactose-1-phosphate, GALT: galactose-1-phosphate uridyltransferase, fGal: free galactose, tGal: total galactose
Data are expressed as mean ± SD or median (interquartile range).

enzyme activity values in this study are still below normal limits, the increase may have an impact on the severity of some long-term outcomes. It is suggested that both the GALT genotype itself and the cryptic residual GALT activity associated with some alleles might impact outcome severity.[11,12] We think that the effect of folic acid on residual GALT activity may be of interest for a hypothesis regarding cryptic GALT activity as a modifier of long-term outcome severity in patients with CG. However, larger and more thorough studies are needed to confirm or refute the significance of these results.

Because the long-term complications of CG have not been effectively prevented, despite dietary treatment, a number of experimental studies have been conducted on the development of different treatment strategies, and some studies are still ongoing. In these studies, galactokinase inhibitors that reduce precursor production,[16] manganese-containing porphyrin compounds[17] and purple sweet potato color[18] that act as antioxidants, and arginine as a stabilizer[19] have been used, but the results should be interpreted with caution, especially given the clinical setting. A study designed to enhance residual enzyme activity showed that GALT enzyme activity was significantly increased after intraperitoneal folic acid treatment compared with the control group.[13] In this study, although GALT enzyme activity increased significantly after the ingestion of folic acid, there was no alteration in the free or total galactose level, probably due to the continuation of a galactose-free diet. Although the mechanism underlying the increased GALT enzyme level induced by folic acid is not known, the increased enzyme level may lead to an alteration of the cellular microenvironment via an epigenetic effect. In addition to diet therapy, a slight increase in GALT enzyme activity may reduce the severity of long-term neurologic and endocrine complications by increasing glycoproteins or galactolipids that contain UDP-Gal. However, all these findings need to be clarified by further research.

The obvious limitation of the study is the lack of a control group. Given the retrospective nature of the study, the data may not have included certain necessary variables (such as a detailed list of foods consumed by the patients). In addition, the sample size is small, and the duration of the study was too short. However, this study has contributed to our knowledge about the role of folic acid in CG patients.

In conclusion, this study showed that folate deficiency, most likely due to poor dietary intake, may develop in pediatric patients with CG. Increasing the daily intake of folic acid may prevent the development of the deficiency. If dietary folic acid cannot be increased, folic acid levels should be checked at regular intervals as part of the follow-up procedure. Folic acid supplementation appears to have a low, but statistically significant, effect on GALT enzyme activity, but comprehensive research is needed to clarify whether there is any clinical significance.

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