Molecular Genetics and Metabolism Reports 26 (2021) 100715

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

journal homepage: www.elsevier.com/locate/ymgmr

Long-term N-carbamylglutamate treatment of hyperammonemia in patients with classic organic acidemias

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ARTICLE INFO

Keywords:
Hyperammonemia
Methylmalonic acidemia
N-Carbamylglutamate
Organic acidurias
Propionic acidemia

ABSTRACT

Background: Classic organic acidurias (OAs) usually characterized by recurrent episodes of acidemia, ketonuria, and hyperammonemia leading to coma and even death if left untreated. Acute hyperammonemia episodes can be treated effectively with N-carbamylglutamate (NCG). The effect of the long-term efficacy of N-carbamylglutamate is little known.

Material-Methods: This retrospective study was conducted at Istanbul University-Cerrahpasa, Cerrahpasa Medical Faculty, Pediatric Nutrition and Metabolism Clinic between January 2012 to January 2018. Patients with classic OAs were enrolled in the study. Patients' ammonia levels, hospitalization needs, hyperammonemia episodes, and management of hyperammonemia were recorded. NCG usage for more than consecutively 15 days was considered as a long-term treatment.

Results: Twenty-one patients, consisting of eleven patients with methylmalonic acidemia (MMA) and ten patients with propionic acidemia (PA) were eligible for the study. N-carbamylglutamate was used as ammonia scavenger for a total of 484 months with a median period of 23 months (min-max: 3–51 months) in all patients. A significant decrease in plasma ammonia levels was detected during long term NCG treatment (55.31 ± 13.762 μmol/L) in comparison with pre NCG treatment period (69.64 ± 17.828 μmol/L) ($p = 0.021$). Hospitalization required hyperammonemia episodes decreased with NCG treatment ($p = 0.013$). In addition, hyperammonemia episodes were also successfully treated with NCG ($p = 0.000$). Mean initial and final ammonia levels at the time of hyperammonemia episodes were 142 ± 46.495 μmol/L and 42.739 ± 12.120 μmol/L, respectively. The average NCG dosage was 85 mg/kg/day (range 12.5–250 mg/kg/day). No apparent side effects were observed.

Conclusion: N-Carbamylglutamate may be deemed an effective and safe treatment modality in the chronic management of hyperammonemia in patients with PA and MMA.

1. Introduction

Propionic acidemia (PA), methylmalonic acidemia (MMA), and isovaleric acidemia (IVA) are autosomal recessive inherited inborn errors of metabolism of branched-chain amino acids and called classical organic acidemias (OAs) [1–3]. Propionic acidemia occurs due to deficiency of propionyl CoA carboxylase and MMA occurs due to methylmalonyl CoA mutase deficiency and both results in impaired metabolism of valine, isoleucine, threonine, and methionine. Isovaleric acidemia is caused by isovaleryl CoA dehydrogenase deficiency in the leucine degradation pathway [1,2,4].

Secondary inhibition of the enzyme N-acetyglutamate synthase (NAGS) due to accumulation of toxic metabolites propionyl CoA, methylmalonyl CoA, and isovaleryl CoA thought to be one of the responsible mechanisms of the hyperammonemia in OAs [5–7]. Impaired Krebs cycle dysfunction, leading to the depletion of glutamine

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https://doi.org/10.1016/j.ymgmr.2021.100715
Received 22 November 2020; Received in revised form 7 January 2021; Accepted 17 January 2021

precursors, and acetyl groups, is also assumed as an another underlying mechanism leading to hyperammonemia [3,4]. Inhibition of NAGS results in the impairment of generating N-acetyglutamate (NAG) which is the activator of carbamoyl-phosphate synthase 1 (CPS1) [3].

These organic acidurias, which are usually characterized by recurrent episodes of acidemia, ketonuria, and hyperammonemia, lead to coma and even death if untreated. In classical form, OAs appear in the first days of life, however, late-onset forms can present at any age. Despite the existence of apparently lifesaving therapy with a low-protein diet, carnitine and emergency treatment modalities, the overall outcome still remains poor [2,4,8,9]. The prognosis is strongly influenced by the duration of coma and hyperammonemia and adequate therapy must be started immediately.

Ammonia scavengers sodium benzoate and sodium phenylbutyrate are the main therapy for the main therapy for hyperammonemia in urea cycle defects. Conjugation of sodium benzoate with glycine to form hippurate and sodium phenylbutyrate with glutamine to form phenylacetylglutamine allow bypassing the urea cycle by offering an alternative pathway for nitrogen disposal through the urinary excretion of hippurate and phenylacetylglutamine. Sodium benzoate is reported to be a safe and effective treatment modality in hyperammonemia treatment in OAs. However, concerns rise about sodium phenylbutyrate treatment because of low glutamine levels accompanied by hyperammonemia in OAs [4,9–13]. Overall, there is a theoretical risk of free CoA depletion in mitochondria secondary to accumulation of MMA and PA therefore use of sodium benzoate and sodium phenylbutyrate in hyperammonemia of OAs remains unclear [4].

N-carbamylglutamate (NCG) is a synthetic analogue of NAG that activates CPS1 in the urea cycle [7]. N-carbamylglutamate treatment is very effective in NAGS deficiency even in lower doses [14]. The safety and efficacy of NCG in acute hyperammonemia episodes of OAs have also been described in various reports [15–18].

This single-centered retrospective study aims to evaluate the impact of long-term NCG usage on hyperammonemia episodes in patients with classical OAs.

2. Material-methods

This single centered retrospective study was conducted at Istanbul University-Cerrahpasa, Cerrahpasa Medical Faculty Pediatric Nutrition and Metabolism Clinic between January 2012 and January 2018. Patients with a diagnosis of classic OAs (PA, MMA, and IVA) were enrolled in the study. Diagnosis of the patients was confirmed with analysis of plasma acylcarnitine profile, urine organic acids, and/or gene mutations. Data regarding the patient's diagnosis, age at the diagnosis, and actual age, plasma ammonia levels, hospitalization needs, hyperammonemia episodes, and management of hyperammonemia were also recorded.

Hyperammonemia was described as ammonia levels above 60 μmol/L, all ammonia levels were recorded. Hyperammonemia episodes were classified as: i) hyperammonemia due to metabolic decompensation ii) mild permanent hperammonemia. N-carbamylglutamate dosages were given milligram (mg) /kilogram (kg) per day. Any additional usage of ammonia scavengers like sodium benzoate or sodium phenylbutyrate were reported. Patients' ammonia levels before/during NCG treatment and the number of hyperammonemia episodes either treated in outpatient clinic or hospitalized were also noted. If extracorporeal ammonia detoxification methods like hemodialysis or hemodiafiltration were performed, ammonia levels and NCG doses of that month were excluded from the study. N-carbamylglutamate usage for more than consecutively 15 days was considered as a long-term treatment. N-carbamylglutamate treatment was given in tablet form divided into three of four doses a day. The usage of NCG was described to each patient before treatment, and patients were regularly checked for the proper usage of the medications. Side effects of NCG were also noted.

2.1. Statistical analysis

The collected data were analyzed by statistical Package for Social Sciences (SPSS) version 21.0 (SPSS Inc., Chicago, IL, USA) and R programming language. Normal distribution of the data was evaluated with Kolmogorov-Smirnov test. The mean, standard deviation, frequency, and ratio values were used as descriptive statistics. A p-value of <0.05 was considered statistically significant. The Wilcoxon test is used for evaluation of data with non-normal distribution.

3. Results

Among 74 patients with IVA, MMA, and PA, 21 patients, who used NCG treatment more than consecutively 15 days, were found eligible and enrolled in the study. Of these 21 patients, 11 patients were diagnosed as MMA and ten with PA. Six patients were male and 15 were female. Patients' mean age was 52.2 months (min-max: 7–146 months). Seven patients had *MMUT* gene, 3 had *MMAB* gene, 3 patients had *PCCA* gene, and 2 patients had *PCCB* gene mutations. Four patient's mutation analysis was not performed due to family issues, and no mutation was detected in two patients (patient 11 and 17).

Two patients received continuous renal replacement therapy at the time of diagnosis, therefore average NCG and ammonia levels were not calculated for that month. N-carbamylglutamate was used as an ammonia scavenger average of 23 months (min-max: 3–51 months) in all patients. N-carbamylglutamate was initiated during the hospital stay due to metabolic decompensation in 11 patients and at the outpatient clinic in 10 patients due to persistent mild hyperammonemia. NCG treatment was initiated at the time of diagnosis in nine patients and within a month following the diagnosis in three patients, due to hyperammonemia. NCG treatment was initiated before 1 year of age in 11 patients.

During the study period, a total of 87 hyperammonemia episodes were treated with NCG dosage adjustment in the outpatient clinic, and 69 were treated by hospitalization.

Data of hospitalization frequency due to hyperammonemia before and during NCG treatment were available in 10 patients. Need of hospitalization significantly decreased during NCG treatment (1.207 ± 1.156/year), in comparison with pre-NCG period (4.321 ± 2.958/year) ($p = 0.013$)

The mean initial and final plasma ammonia levels during the hyperammonemia episodes requiring hospitalization were 142 ± 46.495 μmol/L and 42.739 ± 12.120 μmol/L respectively with a NCG dosage of 148.7 ± 53.204 mg/kg so N-carbamylglutamate treatment was found to be effective on lowering plasma ammonia levels in hospitalized patients ($p = 0.000$).

Data of plasma ammonia levels before and during long-term NCG treatment were available in 11 patients. Plasma ammonia levels decreased significantly following the initiation of NCG treatment (69.64 ± 17.828 μmol/L and 55.31 ± 13.762 μmol/L, respectively) ($p = 0.021$).

The average long term NCG dosage was 85 mg/kg/day, ranging between 12.5 and 250 mg/kg/day. No apparent side effects were observed and none of the patients discontinued NCG treatment. Details of demographic and clinical characteristics are summarized in Table 1. Statistical analysis of the data concerning the mean plasma ammonia levels, dose of ammonia scavengers, and hyperammonemia episodes during NCG treatment are shown in Tables 2, 3 and 4.

Among 21 patients enrolled to the study, sodium benzoate was used in five, sodium phenylbutyrate in one and both sodium benzoate and sodium phenylbutyrate in two patients in addition to NCG treatment. Sodium benzoate was used as an additional ammonia scavenger for an average dose of 181.1 mg/kg/day, for an average of 7.2 months and sodium phenylbutyrate with an average dose of 331 mg/kg/day, for an average of 8.6 months. Addition of sodium benzoate ($p = 1$) or sodium phenylbutyrate ($p = 0.273$) to NCG treatment had no significant effect on lowering plasma ammonia levels. Patient 11 could not tolerate

Table 1
Demographic and clinical characteristics of classic organic aciduria patients.

Patient	Sex	Age (months)	Age of diagnosis (days)	Diagnosis	Genetics
1	F	72	11	MMA	*MMAB gene;* p.R186Q (c.557G > A) homozygote
2	F	91	368	MMA	*MMAB gene;* p.G203RfsX7 (c.607_619delGGAGAGACCGATG) homozygote
3	M	17	244	MMA	*MMAB gene;* p.R186Q (c.557G > A) homozygote
4	F	86	17	MMA	*MMUT gene;* p.D480EfsX7 (c.1440_1444 + 8delinsATCTATC) homozygote
5	M	17	9	MMA	*MMUT gene;* p.Lys54Ter (c.160A > T) homozygote
6	F	146	300	MMA	*MMUT gene;* p.R369H (c.1106G > A) homozygote
7	F	58	780	MMA	*MMUT gene;* p.A141RfsX39 (c.421delG) homozygote
8	M	24	480	MMA	*MMUT gene;* p.L328F (c.982C > A) p.R369H (c.1106G > A) compound heterozygote
9	F	53	540	MMA	*MMUT gene;* p.L328F (c.982C > T) homozygote
10	F	54	19	MMA	*MMUT gene;* p.R103SfsX71 (c.309_327delGCCCTGGACCATCCGCCA) homozygote
11	M	52	153	MMA	No mutation detected
12	F	49	30	PA	*PCCA gene;* p.Cys290Tyr (c.869G > A) homozygote
13	F	23	20	PA	*PCCA gene;* c.69_78del GCAGCTGATG homozygote
14	F	42	31	PA	*PCCA gene;* p.V107M (c.319G > A) homozygote
15	F	111	92	PA	*PCCB gene;* p.Pro208Leu (c.623C > T) homozygote
16	F	63	18	PA	*PCCB gene;* p.I216fs*15 (c.645delG) homozygote
17	M	43	30	PA	No mutation detected
18	F	52	6	PA	Not performed
19	F	7	10	PA	Not performed
20	F	18	21	PA	Not performed
21	M	25	32	PA	Not performed

sodium benzoate treatment due to vomiting therefore only sodium phenylbutyrate was used as an additional therapy. Monthly average NCG dosages and average ammonia levels are given in Figs. 1 and 2.

4. Discussion

Despite the early diagnosis with newborn screening and advanced therapy with protein-restricted diet, carnitine supplementation, and emergency treatments; the overall prognosis in classic OAs remains poor, especially in MMA and PA [2,19]. Hyperammonemia was frequently observed in classic OAs and most often associated with metabolic decompensation that contributes to the risk of neurological damage. The duration of hyperammonemia, abnormal acid-base balance, and the duration of coma correlate with poor neurological outcomes [11,20].

During acute decompensation episodes, the prompt normalization of blood ammonia levels is essential to avoid neurological damage and associated complications [21]. Therefore, first-line treatment in patients with MMA and PA undergoing an acute decompensation includes the reduction of catabolism and the promotion of anabolism by the administration of a protein-restricted high-calorie diet [4]. In case of necessity, ammonia scavengers (sodium benzoate- sodium phenylbutyrate) are used for the treatment of hyperammonemia. Although there are still concerns about these ammonia scavengers; especially low glutamine levels in the case of sodium phenylbutyrate and depletion of acetyl-CoA in sodium benzoate usage [5,9,12,22]. N-carbamylglutamate is widely used for acute hyperammonemia episodes in classic OAs and it is deemed to be safe and effective [5,7,15,16,20]. In our study, the significant decrease in plasma ammonia levels ($p = 0.000$) provided by NCG use in hospitalized patients also supports that NCG treatment is effective in acute management of hyperammonemia.

Several conditions may accelerate metabolic decompensation in OAs, such as infections, vaccination, surgery, etc. Compliance with diet, families, and caregivers paying attention to diet and medical treatment influence the overall prognosis [8,9,19,23]. Metabolic decompensation with hyperammonemia and mild continuous hyperammonemia are the main indications for NCG treatment in our survey.

Long-term NCG usage was previously reported in one retrospective study and one case report. Burlina et al. reported four patients with PA and four patients with MMA for NCG use for 7–16 months with 50 mg/kg/day dosage with favorable effect [24]. The authors find that NCG usage decreased patients' hyperammonemia episodes, overall ammonia levels therefore hospitalization needs. Also, an improvement in protein tolerance. Tummulo et al. reported a decreased number of hyperammonemia episodes as a result of NCG treatment [25].

In our study, the significant decrease in mean plasma ammonia levels before and during NCG treatment in 11 patients ($p = 0.021$) revealed the efficacy of long-term NCG treatment in hyperammonemia in organic acidemias. Also use of ammonia scavengers (sodium benzoate or sodium phenylbutyrate) to NCG treatment, did not have an additional positive effect on lowering plasma levels compared to NCG treatment alone. N-carbamylglutamate was used for a total of 484 months in our study group. The longest consecutive NCG treatment period was 51 months in patient 1. The significant decrease in frequency of hospitalization due to hyperammonemia after initiation of NCG treatment also supports the efficacy of long-term NCG treatment in hyperammonemia in organic acidemias.

N-carbamylglutamate is effective in patients with NAGS deficiency even in lower doses [14]. Studies indicated that NCG could be effective in CPS-1 deficiency and hyperammonemia associated with valproate usage and acute management of hyperammonemia in classic OAs [7,15,16,18,24,25]. The optimum dose is not well defined in OAs. In our study minimum effective dose of NCG is 12.5 mg/kg/day and the maximum dose was 250 mg/kg without any side effects. Classic OAs are ultra-rare diseases, determination of optimum NCG dosage is difficult. Further prospective studies are needed.

In our study, most of our patients' clinical conditions were improved with NCG treatment. However, five of our patients used sodium benzoate, one patient sodium phenylbutyrate and two patients both sodium benzoate and sodium phenylbutyrate in addition to NCG for hyperammonemia. Patient 1 was received sodium benzoate treatment and NCG was added for hyperammonemia episode, later sodium benzoate treatment was discontinued. In patient 2, 14, and 20, sodium benzoate was added to NCG to resolve the hyperammonemia episode. In patient 17, sodium benzoate and NCG treatments were started together in a metabolic attack accompanied by hyperammonemia, but sodium benzoate was discontinued on the follow-up. Patients 10 and 18 were using NCG, sodium benzoate, and sodium phenylbutyrate together because of poor metabolic control in early years of the follow-up. With the repetition of caregivers` education, an increase in diet and medical compliance and good metabolic control was achieved in Patient 18, and ammonia scavengers, including NCG, were discontinued. Sodium phenylbutyrate was added to NCG treatment as an ammonia scavenger in patient 11 due to family issues leading a bad metabolic control. As the patient's care was taken over by the social services, need for hyperammonemia treatment was ended and discontinued.

Table 2
Ammonia levels and dose of ammonia scavengers.

Patient	Initiation of NCG treatment after diagnosis (month)	NCG dosage (mg/kg) Mean (min-max)	Duration of NCG therapy (month)	Ammonia scavenger dose/ Duration of therapy (mg/kg/day)/month		Ammonia levels (mean ± SD) (μmol/L)				
				Sodium Benzoate	Sodium Phenylbutyrate	Before NCG treatment	During NCG treatment	NCG + Sodium Benzoate treatment	NCG + Sodium Phenylbutyrate treatment	Sodium Benzoate treatment
1	$6^{16/30}$	53.8 (30–170)	51	176/4	–	–	54.01 ± 29.668	95.11 ± 35.757	–	105.14 ± 27.536
2	$3^{1/30}$	104.4 (30−200)	20	235/16	–	89 ± 23.060	96.8 ± 43.5	80.41 ± 39.413	–	–
3	$0^{29/30}$	119 (100–125)	10	–	–	49 ± 15.313	51.77 ± 16.237	–	–	–
4	$21^{20/30}$	55.3 (30−100)	15	–	–	95.32 ± 29.392	53.83 ± 23.365	–	–	–
5	$0^{0/30}$	99.6 (70–200)	16	–	–	–	60.35 ± 35.705	–	–	–
6	$91^{5/30}$	43.3 (40–50)	3	–	–	76.92 ± 26.591	37.57 ± 15.523	–	–	–
7	$5^{16/30}$	55.4 (30–80)	23	–	–	56.58 ± 17.634	44.00 ± 18.201	–	–	–
8	$1^{4/30}$	60.6 (50–100)	8	–	–	59.91 ± 21.139	44.74 ± 15.982	–	–	–
9	$4^{26/30}$	53.7 (40–150)	31	–	–	61.79 ± 24.252	49.25 ± 17.303	–	–	–
10	$0^{0/30}$	91.8 (12.5–250)	50	206/7	323/9	–	42.09 ± 16.782	65.69 ± 37.730	65.72 ± 31.119	–
11	$0^{0/30}$	70.9 (30–200)	29	–	380/5	–	46.89 ± 16.895	–	78.75 ± 59.133	–
12	$20^{11/30}$	73 (40–200)	25	–	–	58.82 ± 23.931	54.31 ± 30.339	–	–	–
13	$0^{0/30}$	79 (40–150)	23	–	–	–	56.49 ± 40.161	–	–	–
14	$1^{13/30}$	108.6 (25–200)	37	114/2	–	56.92 ± 19.129	53.27 ± 24.958	41.57 ± 12.528	–	–
15	$91^{21/30}$	65.4 (40–100)	10	–	–	62.00 ± 31.171	60.05 ± 29.788	–	–	–
16	$19^{15/30}$	85.6 (30–200)	42	–	–	–	56.67 ± 30.369	–	–	83.38 ± 43.554
17	$0^{0/30}$	109.7 (25–200)	39	106/3	–	–	56.49 ± 27.115	40.00 ± 17.792	–	
18	$0^{0/30}$	91.8 (50–200)	20	194/6	290/12	89.36 ± 57.462	50.64 ± 12.002	45.60 ± 18.352	61.42 ± 27.361	–
19	$0^{0/30}$	147.8 (100−200)	5	–	–	–	47.13 ± 23.670	–	–	–
20	$0^{0/30}$	162 (100–250)	17	237/13	–	–	87.76 ± 41.765	63.56 ± 32.015	–	–
21	$0^{0/30}$	54.8 (20−100)	10	–	–	–	57.50 ± 26.060	–	–	–

Generally, NCG treatment was well tolerated and no apparent side effects were observed during the NCG treatment. In classic OAs most of the patients suffer from feeding problems [9]. It is very difficult to distinguish whether the nausea/vomiting are due to NCG usage or the disease itself. However, none of our patients complained about consuming NCG, unlike sodium benzoate.

Our retrospective study has some limitations. Due to the rarity of OAs, a relatively small sample sized patients were included in the study. Failure to standardize the conditions in all patients (including concomitant diet and medications, family care) had led a variability in NCG dosage. Also, the NCG dosage could not be standardized in acute versus chronic usage due to the retrospective nature of our study.

In patients in whom the NCG treatment was initiated at the time of diagnosis or within a month, we could not determine whether the need for hospitalization changed according to NCG use. In addition, protein tolerance could not be calculated in patients ≤12 months of age who were breastfed and treated by NCG. Moreover, the frequency of metabolic decompensation decreases with increasing age.

In conclusion, NCG can be a good therapeutic option for long-term hyperammonemia treatment in patients with classic OAs without side effects.

Author contributions

Ertugrul KIYKIM serves as the guarantor for the article. He accepts full responsibility for the work and/or the conduct of the study, had access to the data and controlled the decision to publish. He has been involved in conceptualization, data curation, formal analysis, investigation, methodology, project administration, supervision, validation, writing original draft, writing-review&editing

Ozge OGUZ has been involved in conceptualization, data curation, investigation, writing original draft.

Cisem DUMAN has been involved in conceptualization, data curation, investigation, writing original draft.

Tanyel ZUBARIOGLU has been involved in conceptualization, data curation, formal analysis, methodology, project administration, writing original draft, writing-review&editing.

Mehmet Serif CANSEVER has been involved in conceptualization, data curation, formal analysis, investigation, methodology, project administration, validation, writing original draft,

Cigdem AKTUGLU-ZEYBEK has been involved in in conceptualization, data curation, formal analysis, methodology, project administration, writing original draft, writing-review&editing

Table 3
Hyperammonemia episodes during NCG treatment.

	Hyperammonemia episodes				
	Treatment in outpatient treatment		Inpatient treatment		
	Number of hyperammonemia episodes	Ammonia levels (mean ± SD) (μmol/L)	Number of hyperammonemia episodes	Baseline ammonia levels (mean ± SD) (μmol/L)	Final ammonia level (mean ± SD) (μmol/L)
1	9	106.11 ± 51.246	4	146.75 ± 36.363	43.25 ± 14.886
2	0	–	2	170.00 ± 11.314	48.50 ± 10.607
3	2	64.50 ± 0.707	2	80.50 ± 0.707	49.00 ± 1.414
4	2	83.50 ± 7.778	2	101 ± 15.556	44.50 ± 10.607
5	7	87.57 ± 15.831	3	181.67 ± 98.277	48.00 ± 11.533
6	1	70	0	–	–
7	2	79.50 ± 20.506	0	–	–
8	2	82.00 ± 16.971	2	98.50 ± 6.364	28.50 ± 14.849
9	5	76.60 ± 7.436	0	–	–
10	3	89.00 ± 10.149	6	165.17 ± 40.062	47.00 ± 10.526
11	7	72.86 ± 6.986	2	226.50 ± 123.744	38.00 ± 22.627
12	10	82.70 ± 27.976	6	121.83 ± 28.089	39.00 ± 13.084
13	5	99.80 ± 40.604	4	163.25 ± 62.591	36.00 ± 8.756
14	15	84.20 ± 16.717	6	127.17 ± 29.674	50.5 ± 8.712
15	2	87.50 ± 0.707	2	146 ± 24.042	44 ± 7.071
16	6	83.67 ± 9.873	7	142.29 ± 23.250	39 ± 14.107
17	7	82.71 ± 10.641	7	127.29 ± 22.224	38 ± 14.810
18	2	96.50 ± 33.234	1	249	52
19	0	–	1	90	31
20	0	–	11	145 ± 34.840	44 ± 11.688
21	0	–	1	110	58

Table 4
Statistical analysis of the data concerning plasma ammonia levels according to different treatment modalities.

	Ammonia levels (μmol/L)					p^{υ}
	Before NCG treatment$^{\upsilon}$ (mean ± SD)	NCG treatment$^{\upsilon}$ (mean ± SD)	NCG + Sodium benzoate treatment (mean ± SD)	NCG + Sodium Phenylbutyrate treatment (mean ± SD)	Sodium Benzoate treatment (mean ± SD)	
All patients (N:21)	69.64 ± 17.828	55.31 ± 13.762	65.5 ± 20.077	61.50 ± 16.217	94 ± 15.556	0.021

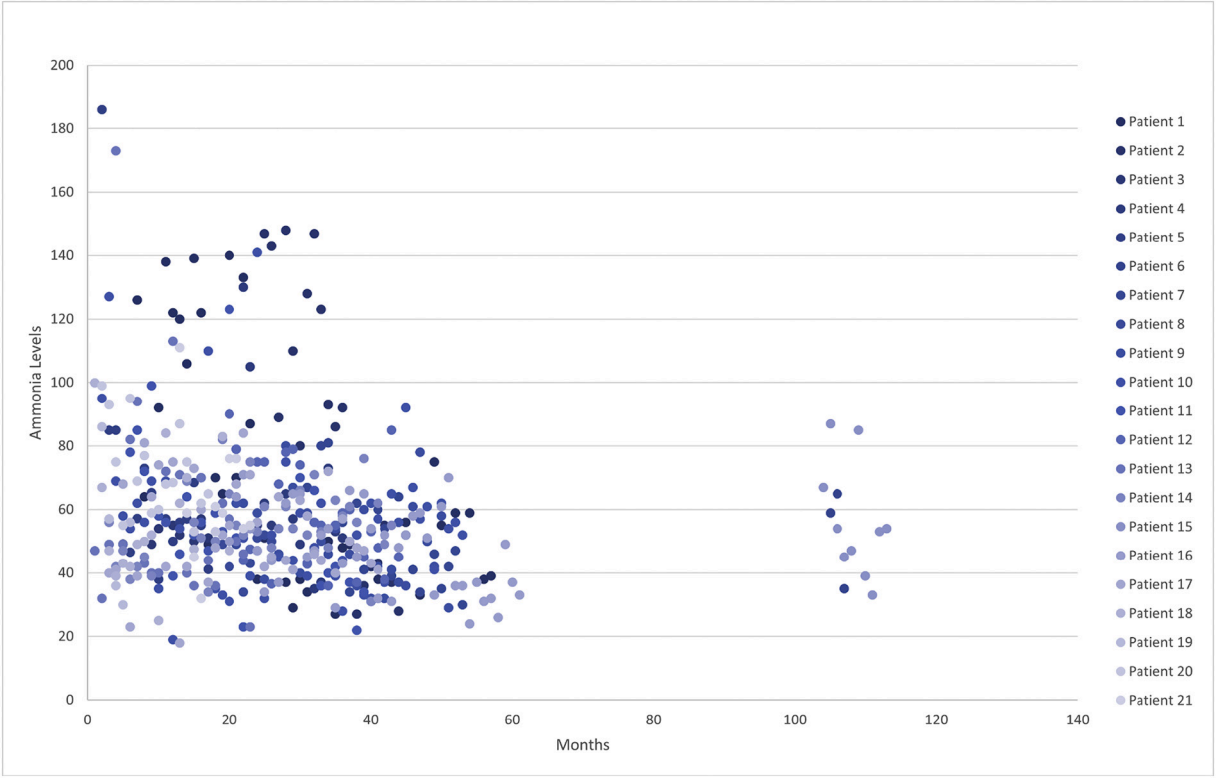

Fig. 1. Patients ammonia levels.

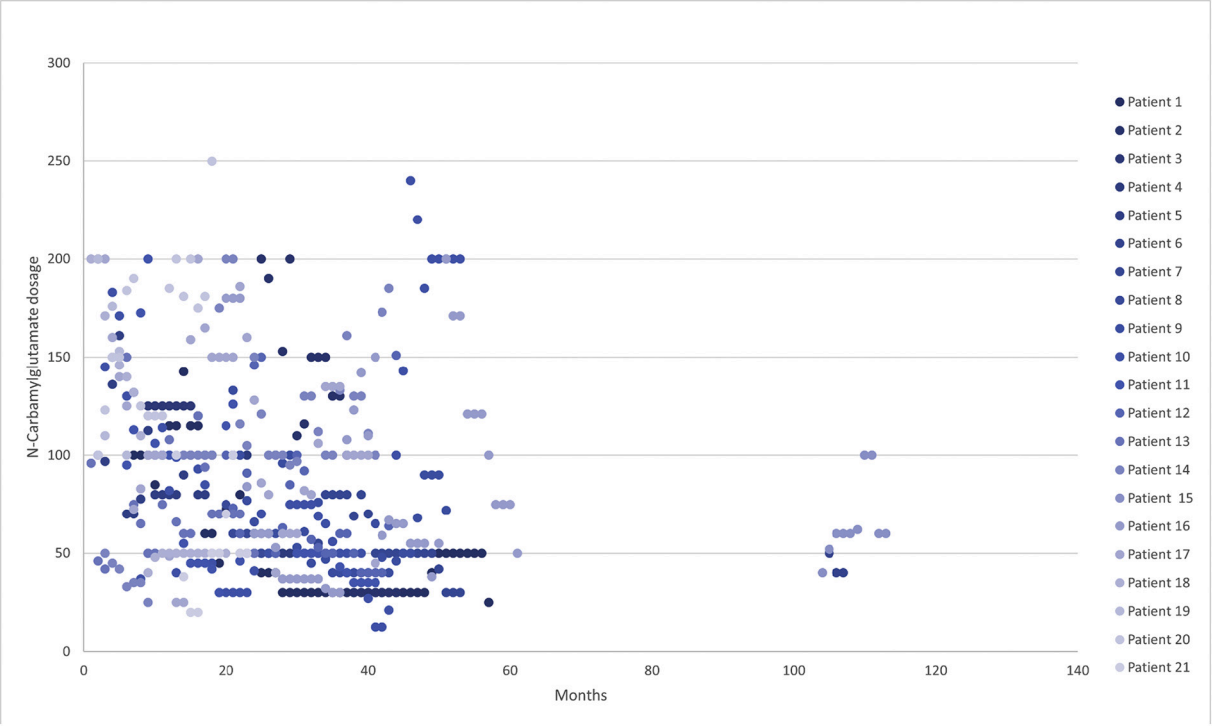

Fig. 2. Patients NCG dosages.

Ethical approval

This study was approved by the Local Ethics Committee of the Istanbul University-Cerrahpasa Cerrahpasa Medical Faculty (protocol: 83045809-604.01.02-, 04/12/2018).

Financial disclosure statement

None.

Funding

None.

Declaration of competing interest

Ertugrul Kiykim, Ozge Oguz, Cisem Duman, Tanyel Zubarioglu, Mehmet Serif Cansever, and Ayse Cigdem Aktuglu-Zeybek declare that they have no conflicts of interest regarding the publication of this manuscript.

The authors confirm independence from their sponsors; the content of this article has not been influenced by its sponsors.

All procedures followed were following the ethical standards of the local Ethical Committee of Cerrahpasa Medical faculty and with the Helsinki Declaration of 1975, as revised in 2000.

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